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CONTENTS

Page

Prof. Dr. Günther Unterstenhöfer:
The Basic Principles of Plant Protection Field Tests 173

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BAYER LEVERKUSEN (GERMANY)
Department for Plant Protection

The Basic Principles of Plant Protection Field Tests

by Prof. Dr. Günther Unterstenhöfer

CONTENTS

Page

Introduction: Substance and object of the field test 174

I. Planning the test and technique of carrying it out 177
1. Fundamental conceptions 177
2. Size of plots 177
3. Shape of plots 179
4. The marginal and adjacent effects 180
5. Number of replications 183
6. Location of plots 184
7. Failures 186
8. Preparation of the test area 187
9. Spraying and dusting 187
10. Test field map and report book 189
11. The conditions for the toxic effect and the secondary effects of the pesticide 190

II. Methods for fixing the date of the test 196
1. Examination of plants for pest infestation 197
2. The trap net and the tree jarring trap method 202
3. Catching insects by means of traps 203
4. Checking flights with the aid of collected pupae 208
5. Observation of phenological phenomena 210
6. Determination of certain meteorological conditions 210
7. Combined practices 212

III. Methods for evaluating the test 215
1. Establishing the number of dead pests and those which survive the treatment 216
2. Determination of infested plants or parts of plants 218

IV. Assessment of effectiveness 223

V. Checking the exactness of the effectiveness of the preparation 230
1. Computation of the mean value and the mean error 232
2. Determining the significance of differences in effect 232

VI. The reliability of the degree of effectiveness established in the field test . . . 234

Introduction: Substance and object of the field test

The possibilities of effectively controlling plant diseases and pests are constantly being improved and extended by the development of new pesticides and control methods. An integrant part of this development is the testing of new control possibilities under practical conditions in the field. The results obtained indicate whether these new control measures, carried out under field conditions which vary from place to place and from year to year, meet with the requirements of farming, namely to give an adequate effect against diseases and pests, without producing any secondary effects which impair the application, raise doubts as to its suitability or even render it impossible. Furthermore, such control measures must not incur high costs. The object of the field test, and also its necessity, is thus established, and its characteristics outlined. If the test is to fulfill its purpose, it must be carried out with absolute precision, and the results must be valid for different soil, climatic and farming conditions over an average number of years. The implementation of such tests designed to give reliable and exact results, involves difficulties which mostly are greatly underestimated because often there is no opportunity to become familiar with the prerequisites essential to an exact test. In this paper, these prerequisites will be outlined fundamently, and explained by means of selected examples. By formulating the biological and technical problem, we shall arrive at the guiding principles necessary for carrying out exact tests. Firstly, the question is raised: What is a test, and what purpose does it serve?

The test is a means of obtaining knowledge. In contrast to mere observations, the test proceeds in that according to the problem to be solved, one or several of the effective factors are changed in a certain manner, and the resultant effects are mostly registered numerically or as estimates. Insofar, the test differs from the observation in which the observer, like the meteorologist, "has no influence whatsoever upon the occurrence" (1).

The result of a test must be conclusive. But the required degree of conclusiveness varies depending upon the object being studied and the task to be fulfilled. For example, in respect of physical questions it is not the same as that required for biological questions.

Generally the purpose of all endeavours to gain information, as established in the substance of the test, is not confined solely to the information itself, but to be able on the basis of the knowledge acquired to predict and, if possible, to steer the course of processes. It is, therefore, intended in the case in question to obtain generally valid principles having approximately the following contents:

When the examined control measures are applied following the occurrence of a certain pest on a certain cultivated plant, it may be expected that with great probability — the exact degree of probability being known — these measures will give successful control of the pest involved, furthermore the damage caused by this pest will be avoided, and the crop will not be harmed either directly or indirectly. In order to arrive at such principles based on the result of tests, it is of the utmost importance to apply the suitable method according to a definite plan. This is essential if each test is to provide a maximum of information. By this, the method is defined as the well-planned procedure for solving a scientific problem.

In order to clarify questions appertaining to plant protection, laboratory and field tests are conducted. The laboratory test is carried out under set conditions. The adjustment of temperature and light, and the elimination of rain and wind produce artificial environmental conditions which, in addition, can be varied in a certain manner. In the field test, natural complex conditions prevail which cannot be influenced by the experimenter. Consequently, the methodical procedure for the field test must be completely different from that adopted for the laboratory test. In the study of plant protection problems, information provided by the laboratory test is supplemented by that obtained in the field test.

During the development of pesticides, the laboratory test is the real research medium. Its main purpose lies in the discovery of new preparations, the determination of such factors as toxic values, mechanism of action and effective range, and the determination of secondary effects on pests, plants and mammals. Furthermore, the most favourable physical properties of the preparation and the likely rates of application are established in the laboratory. In the field test, the efficacy of the preparation is determined when applied at the lowest rate on the most favourable date under practical conditions or conditions similar to those generally prevailing in the field. The field trial, therefore, serves the purpose of examining in practice factors already known. The laboratory test provides the fundamental principles for new controls, and the field test must confirm them.

Due to the fact that the many processes which take place in the field often cannot be explained, it is frequently necessary to return to the laboratory in order to continue the research work so that analyses can be made and causes established. After all, it is in the laboratory that the effect of a certain factor on the toxic process can be examined by keeping all other factors constant by using the technical aids available today, these including air-conditioning chambers, stage thermostats etc. Subsequently, several factors are combined in stages, and their effect determined. In other words, simplified conditions are produced which allow the relationships between certain causes, on the one hand, and observed effects, on the other hand, to be established.

The exact field test differs fundamentally from the so-called demonstration experiment in which normally two equal parts of a plant stand are treated differently and compared, with the differences between the two now and then being recorded numerically or by estimate. For an exact test, it is essential that it is carried out under similar infestation conditions with several replications. In order to conduct such a test at all, several preliminary conditions must be satisfied. The general prerequisites listed by Roemer (2) for conducting exact agricultural field tests also apply in principle to the plant protection test. These are:

1. The personal interest of the experimenter must be so keen that he supervises each process himself.
2. Trained personnel, fully reliable and familiar with the test problem.
3. Efficient equipment, in particular suitable types of sprayers and dusters. It is essential that the exact output of these machines is known.
4. A suitable test site.

Before a test is planned, it is essential to acquire an exact knowledge of the biology, etiology and epidemiology of the pest against which the measures being examined are to be taken, because these factors, in conjunction with the mechanism of action and the other properties of the pesticide, have a decisive bearing upon the test plan. They are also responsible for partially fundamental differences in the technique of test planning, implementation and evaluation existing between the plant protection test, on the one hand, and the field test conducted to clarify questions appertaining to varieties and fertilization, on the other hand. In view of this, the very wide experience gained with respect to tests on varieties and fertilization and the very many directions available on how to conduct these tests, can only be applied to the plant protection test with appreciable limitations.

It is, therefore, necessary to analyze the plant protection field test, and to submit the individual factors which may be influenced technically, to a close study in order to ascertain whether and to what extent the guiding principles which have become established in the field test may have to be amended, making particular allowance for special plant protection factors. Furthermore, when making an exact determination of a reliable effect in the plant protection test, there are in addition factors to be considered, which are characteristic only of this test.

When drawing up a test plan, primary consideration must be given to the size, shape and layout of the plots. The decision on this question will be governed mainly by the marginal and the adjacent effects arising out of the active and passive propagation of diseases and pests. The marginal and the adjacent effects are two factors particularly characteristic of the plant protection test, underlined by the drift of sprays and dusts. The general planning of tests includes settlement of the question as to the number of replications to be made, allowance for failures, and the spraying and dusting technique to be applied.

If recommendations having a wide validity range are to be made on the basis of the results, the guarantee must be given that the results are reliable. To ensure this, the tests must be carried out over a period of several years. During each year, particular importance must be attached to ensuring that the tests are conducted and evaluated at the right time. The correct dates for the tests can be fixed by coordinating the mechanism of action of the insecticide with the "weak spot" in the life-cycle of a pest population. Often this is reduced to very narrow limits. To determine it, use is made of widely varying methods some of which are applied on a broad scale in practice within the scope of the so-called warning service. They include examination of plants for pest infestation, application of methods for catching insects including the use of traps, flight checks with the aid of collected pupae, observations of phenological occurrences, recording of meteorological conditions, and combinations of several research procedures.

For the evaluation of the test, it is very important to collect material for calculating the effect. The selection of the right criterion and its application at the correct time, and suitable sampling have a decisive bearing upon this. Evaluation is carried out according to either the degree of mortality or the extent of damage caused.

The values obtained in the evaluation of the test are calculated to give the degree of efficacy by applying certain formulae. The efficacy indicates by what

percentage a control measure is capable of reducing the rate of infestation or the degree of damage.

In addition to the efficacy, the nature and extent of secondary effects upon the pest, the plant and living beings should be established in the field test, because these factors also have a decisive bearing upon the value of a measure or a preparation.

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I. Planning the test and technique of carrying it out

The statistical examination of test results shows how essential it is that every possible step must be taken from the technical aspect to keep the test error as small as possible, and thus increase the degree of accuracy. This leads automatically to an analysis of the field test, i. e. to analyze it into the different factors which have great bearing upon the accuracy, and to examine what importance is attached to each factor, because in the evaluation the test error is always present. The size of the error can merely be determined within limits by computation, but not changed. Therefore, the greatest importance must be attached to proper planning of the test and carrying out all tasks with extreme care and thoroughness. This, however, is only possible by an improvement of the technique. Under the influence of statistics, the main point of the test method will then have shifted from the evaluation to the planning of the test. Standardized test plans may be regarded as a major result of this development.

1. Fundamental conceptions

When different insecticides (or other types of agricultural chemicals) or plant protection measures or different concentrations of the same product are tested comparatively, each preparation or each concentration is termed as a test unit or variant or given a test number. A test area which consists of one plot for each of the different variants, is called a block.

2. Size of plots

The first step in drawing up a plan for carrying out the test is to fix the size of the plots. In contrast to tests on varieties and fertilization, it is much more difficult to decide on the size of the plot in the plant protection test. Fixed rules cannot be laid down here. On the contrary, various causes arise from case to case, from year to year and from one location to the next, which influence the selection of the plot sizes. Consequently, it is merely possible to give general guiding principles resulting from the peculiarities of the plant protection test, and which may be characterized as follows. It will be shown later that in the plant protection test, the degree of efficacy which is determined according to the rate of infestation or the extent of damage caused, is

selected as a rule in the evaluation as the criterion for the effect of a measure, and it is only in few exceptional cases, and then mostly to supplement the data, that the yield is taken as this criterion. Therefore, the question may be formulated as follows: "To what extent is the rate of infestation or the damage caused influenced, under otherwise similar conditions, by the factor being examined?" "Under otherwise similar conditions" means in this case that in the plots, as the smallest units of a test, in which the efficacy is measured, all conditions influencing the rate of infestation — with the exception of the measures to be tested — are widely uniform and free from fluctuations so that during the course of the test there exists the widest possible causal relationship between the rate of infestation or the extent of damage and the control measure to be tested. However, we know from experience that within the plots there are always differences in the rate of infestation. Their causes are partly of a casual and partly of a regular nature, and are contained in the question on the dispersion of the individual of a species in the biotope (1).

The chance factor plays a decisive part in migration and drift, and is all the more apparent the weaker the rate of infection and, as viewed from the aspect of the individual plants or the different plant parts, the slighter the "probability of contact". Differences in infestation brought about by the laws of nature, are to be found when the causes of a fluctuating infestation density are perceptible in the stands. These include, in particular, specific demands on the biotope (optimum areas), or spatial irregularities in the effect of mortality factors or specific characteristics of the species (oviposition in groups, association of larvae).

Generally speaking, the described state of affairs forces one to the conclusion that the differences in infestation that are always to be expected, will be all the less important the larger the plots, because with larger plots there will be a greater likelihood of sufficient objects being available for the evaluation, with the principle of probability still being maintained for the sampling.

The smaller plot size is of special interest for technical reasons when it is considered that

a) it is possible to carry out the tests on a field scale,

b) it is necessary to eliminate the influence of the individuality of the single pest and the single plant upon the total result, i. e. to obtain results representative of the population.

There is no difficulty in fixing the minimum plot size for testing therapeutic measures designed to control an infestation already present because then the infestation differences can be recorded numerically, and offset by suitable plot dimensions. The main difficulty lies in the testing of preventive methods for which the infestation density and fluctuations in this density cannot be predicted, and consequently cannot be eliminated by means of a known optimum plot size. In this case, we merely have empirical values with a corresponding degree of uncertainty.

The elimination of the individual variability for the single pest and the single plant is attained by carrying out the evaluation on an adequately large number of individuals. For this purpose, a minimum rate of infestation is needed on a minimum number of plants or parts of plants. The accuracy of test results increases proportional to the number of plants or parts of plants,

and the pests parasitizing on them. Whereas the number of plants per unit area depends upon the culture, no direct relationship exists between infestation density and unit area, but is subject, in addition to the aforementioned casual and regular conditions, to the change in population density. The guiding principles valid in agricultural research for breeding efficiency tests with respect to the size of plots — e. g. for cereals 15 to 20 square metres, for peas 20 to 25 sq. m. and for root crops 25 to 30 sq. m. (2) — can be applied in the plant protection test merely for the elimination of the individual variability of the single plant. When there is an adequate population density of soil pests always present in a locality, for example wireworms, nematodes, white grubs, Tipula and potato beetle larvae, it will be possible to adhere closely to these principles. Smaller sized plots will not be selected in the event of yield evaluation being carried out. For field tests, the minimum plot size is generally between 25 square metres (5 × 5 metres) and 100 square metres (10 × 10). For weed control tests, smaller plots of approximately 10 sq. m. (3·3×3 m) in area are preferred in order to make counts, and to be able as far as possible to carry out each test in a similar plant association, a requirement which can be met only with difficulty in large areas (3). When the possibility of an artificial infection exists, plot sizes smaller than those given above can be selected when it is intended only to determine the degree of infestation, provided of course that the adjacent effect — to be discussed later — can be eliminated.

For perennial cultures, too, such as fruit trees and vines, the plot size which here may be taken as being equal to the number of trees or plants, is determined chiefly by the infestation density and the adjacent effect, insofar as the efficacy is selected as the criterion. Even though exact studies of this question are still lacking, past experience has shown that as a rule a minimum number of five trees should be selected. In principle, the plots should be kept as small as possible because large plots cannot be treated as uniformly as small ones. In addition, the evaluation is not so accurate when large plots are used. Furthermore, an extension of the plot size involves more work and material and of course takes up greater space, leading to a restriction of the whole test programme.

3. Shape of plots

In variety and fertilization tests, the shape of the plot has less influence upon accuracy when the test field is fairly uniform. But when this field is very irregular in shape — and this must always be anticipated — it has been established that long, narrow plots give the best results. In the agricultural test, too, the long rectangular shape is given preference over the shorter square shape. Generally, dimensions should be selected whereby one is 5 to 10 times greater than the other. Plots of this shape are also easier to drill. This experience, however, cannot be applied so readily to the plant protection test because the ground differences, as compared with the infestation differences, take a back place as the cause of the irregularity of the plots. There is no proof that with an elongated shape, the infestation differences are more evenly distributed over the plots, which has been proved to be the cause for ground dif-

ferences; in fact this cannot be assumed even when consideration is given to the casual causes of the differences. In plant protection the marginal effect and, to an even greater extent, the adjacent effect take the place of the ground differences as the factors determining the shape of the plots. When spraying and dusting, one is never fully successful in applying the preparation solely on the plots requiring treatment. Even a slight movement of air must be expected to cause drift to the adjacent plots. With elongated plots, this adjacent effect is particularly great. Another adjacent effect with an equal, if not greater influence, is brought about by the active or passive movement of parasites from one plot to the next, which may result in such a marked reciprocal influence that the actual efficacy is concealed. In view of the marginal and adjacent effects, the square shape should, therefore, be given preference over the oblong shape because then a nucleus for the test evaluation is obtained at least in each plot, which has a relatively small error due to the marginal and adjacent effects.

It is in principle a wrong procedure to separate each plot by a small path because as a result of the bigger spacing produced between the peripheral plants and the changes brought about in the microclimatic conditions, variations of unknown magnitude will be caused. Rathermore, the plots should adjoin each other in such a manner that their overall arrangement forms the pattern of a compact stand. Should it be necessary for technical reasons, however, to make a path, it is recommended to leave a separate strip at the edge of the path, which is excluded from the test evaluation.

4. The marginal and adjacent effects

The marginal and adjacent effects have always been known as two factors which have a determining influence on the magnitude of the test error in exact variety and fertilization tests. But these two factors play an even greater part in the plant protection test, and may in fact be regarded as the typical error. Frequently, they completely influence the planning, implementation and evaluation of the test, including the interpretation of the results obtained.

a) Marginal effect

In variety and fertilization tests, a marginal effect is understood to mean that plants growing at the edges of fields or plots give yields differing from those produced inside the stand. The development of the plants at the periphery of the field does not conform with that in the centre of the stand. It is only natural that the influence of the marginal effect upon the plot yields is all the greater the higher the percentage of plants growing along the periphery. In order to establish differences in yield by conducting comparison tests, it is essential that the percentage of plants growing at the periphery is equal in all the plots being compared, or that these plants on the periphery are removed shortly before the harvest, and excluded from the evaluation. As a rule, the latter alternative is taken because in comparison tests on varieties and fertilization, not only the relative but also the absolute yields and yield differences should be established.

The marginal effect is also known in the plant protection test in that the edges of the stand are infested far more greatly than the interior of the stand, especially when there are occurrences of allochthonous pests. Müller (4) gives a most impressive description of the marginal effect and its cause with respect to aphids, based on studies of the colonizing flight. In this colonizing flight as opposed to the long-distance flight, the aphids fly directly above the ground so that they collide with the plants growing on the periphery in a purely mechanical manner, which is the reason why they colonize these plants to a much greater extent than in the interior of the crop. The peripheral colonization therefore takes place especially in compact stands where the migrating aphids not only reach the peripheral plants first but are practically unable to reach the plants growing inside the stand. This explains why the aphid population and the virus infection, too, are often much greater in the rows at the edge of a crop than farther into the field. Müller concludes from this that in crops threatened by aphid attack or susceptible to viruses capable of being transmitted by aphids, it is strongly commendable to avoid making paths between the rows, as far as this is possible, in order to eliminate the peripheral infestation also on the inside edges. Greater infestation may also be observed following occurrences of potato beetles, flea beetles, pollen beetles, spinach leaf miners etc., and the cause is mostly the same. Generally, there is no marginal effect following occurrences of autochthonous pests.

With the fungal diseases, the marginal effect is less marked, apparently because in this case the filter action of the plants at the periphery of the stand is not so pronounced due to the small size of the propagative spores. At all events, there are no indications that the crop is more seriously infected at the periphery than in the interior. At the periphery, however, changes in the microclimate often lead to more unfavourable conditions for infection *(Phytophthora infestans)*. This phenomenon of the marginal effect, which occurs in plant protection tests in addition to varying conditions of plant development attributed to the location, makes it necessary to exclude the peripheries of the crop from the tests. The extent of this exclusion and the way in which the marginal effect is to be rated, must be decided from case to case on the basis of a careful check.

b) The adjacent effect

The adjacent effect is understood to mean the reciprocal influence between adjacent plots. In the variety and fertilization test, this effect is known to vary for the different cultivated plants. As a rule, it is greater in proportion to the difference among the adjacent plots. This effect can be eliminated by sowing covering seeds (protective strips). Generally, strips of one metre in width will be found to be adequate.

Whereas in the variety and fertilization test, the marginal effect is always of greater importance than the adjacent effect, the situation in the plant protection test is just the reverse, and here the adjacent effect is attributed to two causes:

a) the active and passive spread of pathogens and pests,

b) drift of sprays and dusts while carrying out the test.

Explanation to a)

The active and passive spread of pathogens and pests, migration, flight, propagation by rain, wind and living organism including human beings (e. g. when inspecting and working in the plots), apply to most pathogens and pests whereby the extent, distance and direction of spread may vary most considerably from species to species and depending upon the environmental conditions. As a result of these possibilities of propagation, there are continual changes in the density of infestation on a more or less large scale, which make the evaluation of the test difficult insofar as there is no causal relationship between them and the changes in the degree of infestation brought about by the control measures being tested, added to which both forms of changes occur simultaneously. The adjacent effect is, therefore, of importance to the evaluation of the test in that it renders difficult the appraisal of the evident causal relationship between a measure being tested, on the one hand, and the infestation density, on the other hand, in other words the determination of the efficacy.

Fundamentally, a distinction can be made between two causal adjacent effects which require appraisal, and which are particularly evident with occurrences of aphids.

1. The large-scale adjacent effect or distant effect caused by distant infection. These infections are brought about, in the case of aphids, by allochthonous winged insects on their long-distance flight, causing an adjacent effect over large areas, whereby the distance between the starting point and the colonizing point is great and also unknown. It takes place chiefly in the direction of the main wind, and is the cause of saltatory propagation, in particular, of persistent viruses (yellows, leaf roll). It is a factor which is unalterable, and which cannot be eliminated either completely or partially. This adjacent effect is not known as a source of error in the variety and fertilization test.

2. The small-scale adjacent effect caused by close infections. It is brought about by autochthonous wingless aphids and by allochthonous alate aphids on their colonizing flight, causing an adjacent effect over short distances, and being responsible for the fluctuating spread of persistent and non-persistent viruses (mosaic of beets, Y-virus of potatoes). This effect can be eliminated for aphids by

   a) excluding the peripheries of the plots,

   b) sowing protective seed in the form of dense drilled rows, 15 cm wide, using oats, for example, which surround each plot.

Adjacent effects over large and small distances occur on a marked scale with insects (winter moth and gypsy moth larvae, beetles, butterflies, flies) and fungal diseases (anemochoria). Their influence is reduced by selecting sufficiently large square plots, and by evaluating the inner nucleus of these plots.

This constitutes an interesting sphere for systematic research.

Explanation of b) D r i f t

Drift of sprays and dusts from one plot to another takes place while the test is being carried out, on a more or less large scale depending upon the

velocity of the wind. Even when one conforms with the general rule of conducting tests only at wind velocities of one to two metres per second, drift to one of the adjoining plots may nevertheless be expected. Generally, drift bears the character of an adjacent effect over a small distance. An attempt should be made to eliminate it by setting up protective screens made of cloth around the plots. This practice will prove successful with field crops, low trees and bushes but not with tall trees, for example in fruit farming. In the latter case, the only alternative is to select larger plots and to exclude from the evaluation those edges of the plots affected most by drift. Particularly great difficulties are experienced with strongly volatile active ingredients, e. g. certain weed killers. Such types of active ingredients make themselves noticeable especially on test fields where different test problems are continually being dealt with in a small area.

It is extremely important to take into consideration the marginal and adjacent effects if the test results are to be interpreted correctly. Above all, a careful check must be made establishing to what extent results obtained from tests carried out in small plots can be applied freely and without limitation to large-scale practical application, where under certain circumstances the close infections are completely lacking or exist only to a lesser degree due to untreated checks.

5. Number of replications

In order to register the magnitude of the test error, it is necessary to arrange for replications. Proof has been furnished that the accuracy of the results can be improved by increasing the number of replications. But on the other hand, it is known that by raising the number of replications, the improvement of accuracy cannot be increased proportionally but becomes progressively smaller whereas there is a constant increase in the work involved and the time taken. Allowance must be made for this when deciding on the number of replications to be carried out. In order to run field tests on sound economical lines, every effort should, therefore, be made to keep the number of replications as low as possible.

It is not possible to give a generally valid ruling on the minimum number of replications. When planning the tests, the number of replications needed should be decided from case to case after careful deliberation, and in doing so consideration should be given to the following points:

1. The anticipated differences in effect.
2. The standard to be applied for assessing the efficacy.
3. Uniformity of the infestation and the soil.
4. Density of infestation.

Mostly, rough indications of whether the anticipated differences will be large or small are gained on the basis of laboratory tests or initial exploratory tests. The smaller the expected differences the greater will have to be the selected number of replications, and inversely when the differences are larger the

number of replications can be proportionally smaller, irrespective of whether the yield or the efficacy is taken as the criterion.

When the yield is used exclusively as the standard, the variability of the crop type on which the test is carried out, will continue to determine the number of replications because the various types react differently, as established, to the fertility of the soil and differences in injury. As a rule, the productivity of spring cereals and hemp is greatly affected by environmental influences, whereas that of winter cereals and potatoes is less affected.

Uniformity of the infestation and the soil continues to determine the number of replications, in fact to such an extent that this number may be all the smaller, the more uniform the conditions.

Finally, the density of infestation also has a bearing upon the number of replications to be carried out. The weaker the infestation, the greater this number must be, so that sampling can be made from a sufficiently large number of measurable entities, at the same time maintaining the casual sampling.

The question of the number of replications to be carried out, shows that the practical possibilities in the end have a decisive influence upon this. Under very favourable conditions, three replications will do. Generally, four to five parallels are sufficient. Should it be required to establish fine differences, up to six or eight replications will be needed. It will only be possible and necessary to go beyond this level in exceptional cases.

6. Location of plots

The modern test systems have been developed by R. A. F i s c h e r and his school. Nowadays they have replaced the old methods. Several basic features are peculiar to all these plans. The plots are casually distributed within a block. In order to allow well-balanced influences to be exerted on the different test plots, the blocks are replicated. In this, it is assumed justifiably that the probability of the same influences (pest infestation, growth conditions) being exerted on the different test plots is all the greater, the larger the number of replications carried out, because similar conditions occur repeatedly in the vicinity around the block. In the test field, the blocks can be distributed at random.

1. Block arrangement

Block								
4	4	6	5	8	3	2	1	7
3	3	5	1	6	8	7	4	2
2	8	2	3	4	1	7	6	5
1	1	2	3	4	5	6	7	8

Test with 8 test plots in 4 replications. Each block contains all test plots once. Test plots distributed at random in block.

2. Latin quadrate

Block \ Column	2	4	5	1	6	3
3	6	2	3	5	4	1
5	4	6	1	3	2	5
2	1	3	4	6	5	2
6	3	5	6	2	1	4
1	2	4	5	1	6	3
4	5	1	2	4	3	6

6 × 6 Latin quadrate

Each test plot must appear once in each block and in each column. Elimination of block differences in the block and in the columns.

Columns and blocks distributed at random.

No. of replications = No. of test plots.

Limited to tests with few (4 to 8) test plots.

3. Latin rectangle

8 test plots in 4 replications in 4 × 2 Latin rectangle

	Column 1		2		3		4	
4	5	8	6	7	2	4	1	3
3	6	3	8	2	1	7	5	4
2	4	7	5	1	3	8	2	6
1	1	2	3	4	5	6	7	8

Columns are formed covering the width of several plots; the long columns of the Latin quadrate are, therefore, "buckled".

No. of test plots must be a square or divisible by 2.

Like the Latin quadrate, this arrangement has the advantage of the twofold grouping, and can be used for larger numbers of test plots.

4. Factorial block arrangement

Block	1			2			3			4		
	11	12	13	21	22	23	12	21	23	11	13	22	13	12	21	23	22	11	23	11	13	12	21	22

2 × 3 tests with several replications. Test with several factors.

Example: Several acaricides applied at different times

1st figure = time of spraying
11 = pre-blossom, "Eradex"
12 = pre-blossom, "E 605"
13 = pre-blossom, "Systox"

2nd figure = spray preparation
21 = post-blossom, "Eradex"
22 = post-blossom, "E 605"
23 = post-blossom, "Systox"

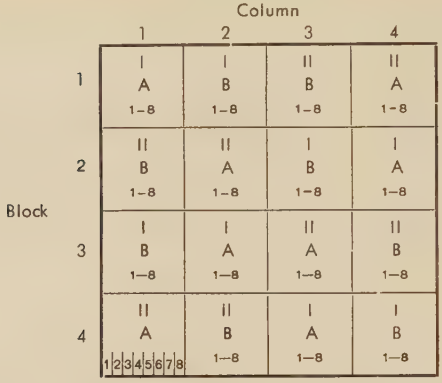
5. Factorial arrangement in Latin quadrate

Block \ Column	1	2	3	4	5	6
1	23	13	22	11	21	12
2	21	23	12	22	13	11
3	12	22	21	23	11	13
4	22	11	23	13	12	21
5	13	21	11	12	23	22
6	11	12	13	21	22	23

2 × 3 tests in 6 × 6 Latin quadrate

Principle of twofold grouping in test plan

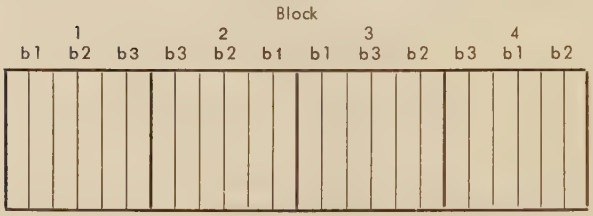
6. Factorial arrangement in Latin rectangle



2 × 2 × 8 tests

2 dosages (I, II) at 2 times (A, B) of 8 preparations (1 – 8)
To be used when number of test plots is too large for Latin quadrate

7. Split plot



2 × 3 tests according to split plot (cf. 4 and 5)
b1, b2, b3 = preparation a1, a2 = time of spraying

Principle of split plot: a part of the one factor is split in order to allocate over the partial areas the steps of the other factor.

7. Failures

Failures have an influence upon the test result especially when the yield is selected as the criterion for the efficacy of a preparation. They make it necessary to correct the plot yields. For this purpose, several suggestions have been put forward. As the plants adjoining the failures produce yields above average due to more favourable growing conditions, these are removed before the harvest, and the yields are corrected in relation to the normal plants. This procedure, however, is an object of controversy but is nevertheless satisfactory for practical purposes. In agricultural research, it is adjudged possible for the covariant analysis to be corrected exactly. In plant protection research, cases in which plants are not attacked must also be regarded as failures. This is applicable, for example, when a fruit tree produces no fruit or when it loses the fruit prematurely so that an evaluation cannot be carried out in studies on the effect of preparations applied against fruit scab or fruit maggots. When the efficacy of the preparation is taken as the criterion, the deficiency of

objects on which the measurement should have been made, will therefore generally be regarded as a failure. Such failures can only be prevented by selecting sufficiently large dimensions for the plots. When the density of infestation is below a certain level so that it is not even possible to carry out at least 30 measurements, the result obtained will have to be regarded as much too fortuitous. Such results cannot be used to draw convincing conclusions.

8. Preparation of the test area

Once careful studies have shown that a selected test area is suitable for its required purpose, it should be ensured when planning the test that all important test conditions are uniform. Fertilization and cultural practices including pruning of fruit trees should be carried out at the same time and in the same manner in order to avoid differences in the preparation. Whenever possible, stable manure should not be spread on the field in the year the test is carried out.

Liquid manure and green manure should not be applied especially on annual plants. Should it prove absolutely necessary to use stable manure, then the quantity to be applied to the whole area should be mixed thoroughly beforehand, and spread evenly. Furthermore, use should only be made of well rotted stable manure.

Artificial manure should be spread in the direction that the wind is blowing. Paths and peripheral strips that may be laid out, should kept free of weeds.

Particular importance should be attached to compliance with these directions because a test should allow recognition not only of differences in efficacy but also of secondary effects and, if necessary, influences on yield. It must, however, be possible to ascertain whether these effects are causally related to the test problem, and not caused by other factors.

In preparing the test area, the experimenter is able to a certain extent to bring influences to bear upon the rate of infestation. For example, late sowing of beets will give more serious infection by yellows. Furthermore, by cultivating the plant to be studied in the same field over a period of several years, infestation by certain pests (nematodes) can be increased, and a higher degree of injuries brought about by cultivating susceptible varieties.

9. Spraying and dusting

In order to conduct tests quickly and faultlessly, it is essential that all tasks are carefully prepared and carried out with a maximum of precision. The possibility of errors being made commences when the preparations are weighed and measured off. The quantities of preparations stipulated for the different plots must be weighed out exactly, and then placed in glass containers or bags which are labelled giving details of the contents, quantity and plot number. To avoid errors at this stage, the prepared quantities should be checked to ensure they are correct.

In addition to having available the required equipment and machines which should be checked beforehand to make sure they operate satisfactorily, and tools to make quick repairs and remedy minor faults such as clogged sprayer nozzles etc., provision must be made for adequate supplies of water to be available in

the test field. Facilities must also be provided for all personnel to wash themselves.

As a rule, sprays are prepared with a little water. Once this has been done, a few litres of water are poured into the sprayer. Afterwards the stock solution is poured in through a strainer, and the bulk of water is then added. Any residues of stock solution adhering to the vessel in which the spray is first prepared, should be rinsed out with water and emptied into the sprayer tank. The dilution must be mixed thoroughly.

The next task is to distribute the preparation evenly in the plots, and the efficiency with which it can be done depends largely upon the weather. Wind, sunshine, rain and relative humidity are all factors which have a bearing upon the distribution of active ingredient.

a) The influence of wind

If they are to become fully effective, the fine, suspended particles of dust preparations must settle on the plants and pests. Even a slight wind will cause a part of the dust to be carried away from the zone where it is supposed to act. Dust settles best in calm weather, although it is most evenly distributed when a slight wind is blowing. In other words, the wind assists the distribution of dust but hinders it from settling. Generally, a wind velocity of 1 metre per second is the most favourable for dusting. But dust applications can also be made at wind velocities of up to 2 metres per second when using high dosages in large plots to give control of very susceptible parasites.

Attention must be given to the direction of the wind when making the application. Spraying and dusting should be carried out at a right angle to the direction of the wind, beginning for example on the east side when a west wind is blowing, after previously treating the north and south peripheries. The operator, as a result, is practically always outside the range of the spray mist or dust cloud, and does not pass through the treated zone, either.

b) Sunshine

Usually, after the sun has warmed the ground, rising currents tend to prevent the dust from settling on the plants. On the other hand, heat generally accelerates the rate of mortality, but may even slow it down on occasions.

c) Rain

In unsettled weather, rapidly acting preparations should be used, but only when the prospects appear sufficiently favourable to allow the preparation to act for at least a few hours. Stomach poisons should not be used in unsettled conditions because they mostly take a longer time to act. After rain has fallen, spraying and dusting operations should not be started until the plants have dried.

d) Relative humidity

High relative humidity mostly increases the efficacy of preparations. When conditions are too dry, it has been found from experience that the efficacy, in particular the rate of the effect, is lower because of the reduced feeding activities of many pests, e. g. the adult potato beetles appearing in Spring.

With fruit trees, and especially standards, it is difficult to satisfy the requirement of having to treat thoroughly and evenly all parts of the tree infested by pests. This is particularly the case when trial controls are carried out in the vegetation season against pests like San José scale and woolly aphid which colonize the branches and shoots. At this time of the year, the foliage prevents penetration of the preparation to give the bark a good wetting. According to C r e u z b u r g (5), about two-thirds of the bark surface in the top section of the tree, are wetted at the most. But even in Winter, coverage is often inadequate, particularly on shoot tips and on the underside of low, horizontal boughs.

The importance of giving the tops and sides sheltered from the wind, a uniform treatment is evident from observations which show that fruit maggots, for example, apparently prefer the tree tops (6), in other words parts which are difficult to treat and are exposed to rain resulting in the preparation being washed off. Furthermore, winter oviposition of the winter moth (7) also takes place primarily in tree tops.

F r i e d r i c h (8) points out that insects avoid that side of branches facing the wind, when they lay their winter eggs. The inactive adult scale insects also occur chiefly on those sides sheltered from the prevailing wind. This point must be observed when applying winter sprays because the general policy is not to spray against the wind, in other words from the sides sheltered from the wind and which are most heavily colonized.

The working methods of different persons mostly vary, thus having an influence on the result, and therefore one and the same person should not always treat the same plots. Arrangements should be made for the spraying team to be changed round, just like the replications. Furthermore, only one plot should be treated with one tank filling, and the practice of using a large filling to treat several plots should be avoided, otherwise the rates of application in the different plots fluctuate too greatly.

Sprayers and dusters must always be given a thorough cleaning after use, and before they are filled with a different preparation. Sprayers should be rinsed out with clear water to which soda is added if necessary. This task can be completed more quickly by unscrewing the nozzles.

10. Test field map and report book

The test plan is generally drawn up and all preparations made during the winter months. As a result, much time is saved, and there is ample opportunity to make a careful check to ensure that nothing has been left unconsidered which might be of importance for obtaining an exact clarification of the test problems. Tests which are improvised and not carefully planned always give unreliable results.

The complete test plan contains all details of the test such as the layout of the test field, varieties, fertilization, previous crop, type of soil, type of pesticide to be tested, rates of application, dates of application etc. These details are contained in neat order in a field test map or a report book in which records are entered daily. During the course of the test, these details should be supplemented regularly by exact descriptions of the measures carried out and the observations made, together with the dates and the prevailing weather conditions. The observations should not only cover the actual test problem but

should also be extended to the entire development of vegetation including details on the occurrence and incidence of other pests and diseases, night frosts and other abnormal weather conditions, failures, parasitization etc. The totality of these factors in the end determines the result of the test, and enables a causal interpretation to be made of it. There is no end to the benefits profited from the registration of observations. After all, each field test primarily constitutes a collection of material, and the more that is available on completion of the test, the more lucrative will be the interpretation of the values obtained, added to which far-reaching conclusions can be drawn.

11. The conditions for the toxic effect and the secondary effects of the pesticide

Apart from the actual test problem which must be observed and registered in all details, careful consideration must also be given to two other factors:

1. The conditions for the toxic effect
2. The secondary effects

On 1) The conditions for the toxic effect

The level of efficacy is not absolute but is governed by different conditions. The most important of these include: the disposition of the organism and its stage-specific susceptibility, the environmental conditions, the season, the growth vigour of the plants and the physical properties of the preparation.

The disposition of the organism and its stage-specific susceptibility

An analysis of the infestation symptoms, in which it is of great importance to determine the disposition of the population, is made before the test is carried out in the case of therapeutic measures, and by observing the untreated check in the case of prophylactic measures. The disposition of the organism and the population towards poisons differs according to the stage of development, age and influence of such external conditions as state of health, parasitization, food etc. With insects, the differences between the various stages of development are often not only gradual but sometimes even of a fundamental nature. Freshly emerged larvae are as a rule more susceptible than older instars. The resistance of insects immediately prior to pupation may increase to such an extent that they are practically immune to the concentrations considered for application; this has repeatedly been established for potato beetle larvae and several moth caterpillars. In this, the differences in the effect of the various preparations are not alike, and are, therefore, of extreme importance when characterizing their potency. But often there are also marked differences within a single stage of development. The susceptibility of larvae shortly after ecdysis is greater than shortly before the moult. Young pollen beetles *(Meligethes aeneus)* are more resistant than the adults of this species (9). Even in the case of potato beetles *(Leptinotarsa decemlineata)*, the overwintering beetles of the preceding year's generation react differently from the adults of the summer generation (10). For this reason, precise records must be entered at the start of the test and in the evaluation of the test, on the state of a population and the development stages to which the surviving and dead insects belong, especially as all stages of development often occur at the same time in the field. Widely varying degrees of efficacy may be obtained depending upon the

state of a population at the time the poison becomes effective. In addition to the age, the state of health and the degree of parasitization may also have a decisive influence upon the intensity of the effect. It is, therefore, essential in doubtful cases, to establish whether there is a possible predisposition in this connection by studying an appropriate number of insects.

Environmental conditions

Reference has already been made to the importance of the weather conditions with respect to the application, uniform distribution and effect of sprays and dusts. Furthermore, they determine the annual error which has still to be discussed, and have a decisive bearing upon the reliability of the results. The weather conditions constitute an influential factor from two aspects, in that on the one hand they determine the disposition of the pests and the plants, and on the other hand they influence the insecticidal potency of the preparation. Heavy rainfall and wind reduce to a lesser or larger degree the action of the active ingredient by washing off or blowing away the deposits of preparation, whereby the extent of this reduction is largely determined by the grade of the physical properties of the preparation. The exact determination of this reduction constitutes an important part of the field test.

Detailed studies have revealed that the rubbing together of leaves and fruits and the brushing effect they produce on touching each other, have a great bearing upon the decrease in the amount of active ingredient originally deposited on these parts, and that these effects (rubbing and brushing) often attach greater importance than rainfall.

Time factor

A distinction is made between a time-associated and a time-free toxic effect. A toxic effect is free from the time factor when a certain absolute toxic dosage is required to give a toxic effect. On the other hand, the toxic effect is bound up with the time factor when a relationship exists between the poison concentration and the time for which the rate of mortality is the standard. The time factor is a very important one insofar as it is desired that the poison becomes active as quickly as possible in order to preclude injuries to the plants, and also to minimize the relation between the toxic effect and sudden changes in weather, in other words to eliminate as far as possible any influence such changes may have upon the effect. When a poison acts rapidly, the influence of sudden bad weather conditions at least on the direct effect will be proportionally less, and the application will constitute a correspondingly smaller risk for the farmer. For this reason, the rate at which the preparation becomes effective must also be determined in the evaluation of the test.

The growth vigour of the plants

In all cases where the efficacy of preparations is to a great extent determined by the residual effect — due to the prolonged flight of pests or persistent infection (e. g. in the case of fruit maggots and apple scab, *Venturia inaequalis*) — a part is played not only by the "stability" of the preparation and the weather but also to a very wide extent by the growth vigour of the plants. This fact is often completely neglected or else underestimated. Studies conducted by F a h e y and R u s k (11) on apples of the Grimes Golden variety to establish increases in the surface area of the fruit between petal fall and harvest-time, have shown that there is a daily increase in surface area of

1 sq. cm. The relative increase in growth is very great particularly during the first few weeks of development, and in the first few days it amounts to values of up to 100 %, only to become progressively smaller later. Due to this increase of the surface area of the fruit, the concentration of active ingredient present on it is constantly diluted, the degree of dilution being proportional to the relative growth. The above authors proved that in the first 45 days following the blossom period, sprays carried out at intervals of ten days were not adequate to give new growth satisfactory protection. Similar results were obtained by Hamilton (12) who also studied leaf growth as well as fruit growth. He found that the growth of the foliage is practically completed at the end of June to the beginning of July, and that the highest rate of growth is in May. Comparative studies conducted by Stanley (13) on tobacco and kidney beans revealed that the daily increase in leaf area is approximately 13 % for beans, and roughly 7 % for tobacco. He also made the important finding that despite the varying growth and the different size of both plant species, the leaf area per unit area of planted field is practically the same, being approximately five times as great.

With respect to the implementation of the test, this means that full allowance must be given for the growth vigour of plants when deciding on the intervals at which sprays are to be applied, and when interpreting the results. An exact record must be kept of the state of the plants when the test is started, and while it is being conducted. The results obtained for example in trial controls of the first generation of fruit maggots may differ appreciably from those recorded in the control of the second generation. The same applies to the control of apple scab and other fungal diseases, where frequently better results are obtained at the end of the main growth period than at an earlier stage.

The physical properties of the preparation

Apart from being dependent upon the material composition and the dosage, the efficacy of an agricultural chemical used for the control of pests and diseases is governed largely by the physical properties of the preparation. Even though a thorough examination of the physical properties of a finished product is made in the laboratory, the critical rating is carried out in the field. In the main, the preparations applied in the field are sprays and dusts. Use is also made of fumigants although almost exclusively for the control of soil pests.

Important properties of spray preparations include a good suspension property of the particles in water, good wetting power of the wash, and resistance to rain of the spray deposit. An essential requirement of dusts is that they atomize readily to form a good dust cloud which ensures proper distribution so necessary for controlling less mobile insects. Coagulation and lump formation of dust particles are undesired. Good adhesion and resistance to rain are important properties insofar as deposits of preparation less influenced by wind and rain when on the plants, give promise of better and more reliable effectiveness.

Fumigants must possess good spreading and penetrating properties.

The physical behaviour of test preparations must be observed very carefully when preparations containing the same active ingredient are subjected to comparison tests in which the anticipated differences in efficacy are determined largely by the physical properties.

On 2) The secondary effects

In order to make an assessment of the practical value of an agricultural chemical, it is not enough merely to have a knowledge of the efficacy and the conditions under which it is attained. On the contrary, it is also of the utmost importance to know the so-called secondary effects which automatically accompany the application of the preparation. Secondary effects are understood to mean all effects which occur in addition to the actual purpose for which the preparation is used, namely to afford the plants protection by killing the pests, or expressed generally, by destroying the relation between parasite and host. As a rule, secondary effects are unintentional effects exerted on the pest, the plant, other animals and human beings. They may be desirable, insignificant or harmful. Their exact registration is part of the test evaluation.

Secondary effects on the pest

A 100 % kill of pests is very seldom obtained in field tests. The reasons for this may be of a technical nature associated with the methods of application, or they may be due to the fact that the applied concentration or the rate of application is too low so that the most resistant members of the population are not killed. It is, therefore, an important task to establish which of the two above factors is to be held responsible for the failure to obtain a 100 % control, or in other words for the survival of certain individuals. After all, the development of resistant biotypes may be directly connected with this. Such development constitutes a phenomenon long observed among numerous species of pests (14, 15). Particularly acute resistance to insecticides is understood to mean that insecticides which have been applied with success against a certain pest over a more or less extended period, become progressively less effective, until in the end they are completely useless for the purpose involved. The significance of this resistance to insecticides lies in the fact that it determines the service life of a preparation when the technical progress made in the development of a preparation is disregarded.

An exact analysis of the infestation symptoms may provide valuable information as to whether it is a case of the efficacy of a preparation being generally inadequate or whether due to technical inefficiency during the spraying or dusting operation, it is only in certain places that insufficient quantities of the preparation were able to act. Generally, it may be assumed that the effect of the preparation or the applied concentration is inadequate when pests which have survived the control are to be found evenly distributed throughout the plot, in other words when the conclusion is justified that mortality has not been caused despite contact between pest and poison. If on the other hand, surviving insects are only to be found in small isolated groups, then it must be concluded that the preparation has not been spread properly.

An exact description of the infestation following application of the preparation will provide valuable explanations on the efficacy.

Secondary effects on the plants

The growth of plants may be harmed or stimulated by the action of chemicals applied to control pests and diseases. A positive influence is exerted when the productivity of the plants is increased, in other words when stimulation of growth is caused or when the preparation acts as a nutrient, such as described, for example, in respect of carbon disulphide or several organic phosphorus

compounds. Such effects are made manifest by a better growth of the plants, improved formation of chlorophyll and larger yields. When the efficacy is otherwise the same, preparations having a favourable influence on plants are given preference.

Plant injuries are observed more frequently. One speaks of scorching when tissue parts on leaves and shoots are caused to die off (necrosis), appearing as spots, discolorations and dried-up parts. The extent of the damage is expressed in ratings (0 to 5).

Injuries do not always appear in the form of necrosis but also as abnormal growth or poor formation of chlorophyll. The symptoms are then mostly characteristic. They must be described exactly, and importance should be attached to the nature and extent of the injuries, and their effect on the yield.

Injuries which affect the flavour of the crop are easily overlooked and their cause is mistaken because mostly they are without symptoms during the vegetation period. The assessment of a preparation should at all costs include a taste test of the crop after it has been harvested.

Injuries to plants caused by chemicals are often closely associated with the variety and the climate. Injuries inflicted by copper are observed more frequently in wet districts and in moist weather, whereas sulphur injuries appear particularly in dry and warm regions, or at high temperatures and low relative humidity.

Secondary effects on animals and insects

Since all preparations that are applied have a more or less wide range of action, allowance must always be made for the preparation being effective on other organisms, whether they be harmful, unimportant or beneficial. It is very important to establish whether the application of a preparation constitutes a danger to bees. This is all the more necessary when the date fixed for the application is close to the blossom period, or when there is every likelihood of weeds being in bloom at the time the crop is treated. When it is not known what harm the preparations being tested may cause to bees, then they should not be applied under any circumstances whatsoever in flowering crops. At all events, it is advisable especially when carrying out large-scale trials, to inform all bee-keepers in the vicinity, and to keep their bee stocks under observation. In addition to bees, other beneficial insects, in particular the natural enemies of the pests, should also be included in the observation. This is done on large plants by spreading out trap nets, and in the case of small field crops by recording the number of dead insects found on the ground. The extent of the secondary effects on beneficial insects — it is in the interest of plant protection to preserve these insects — differs greatly for many preparations. Even though nothing definite can be said about the consequences arising out of the elimination of insects classified as beneficial or insignificant, use should be made primarily of those preparations having the weakest secondary effect, that is when the conditions are otherwise the same. The increase in the occurrences of spider mites in fruit farming, which may be attributed at least partly to the application of preparations with a wide range of action so that they eradicate or greatly reduce the numbers of predators, emphasizes how very important it is not to neglect the effect of new preparations on the biosociology.

Other important organisms living outdoors are game and fish which may also be exposed to great hazards following applications of strong poisons.

Mostly, fish are more seriously threatened than game. Water contaminated with active ingredient, which is carried away in rivers and streams from the treated area, especially following controls in forests, has sometimes led to large numbers of fish being poisoned. The same applies to cattle, sheep, poultry etc. It is extremely important that proper precautionary measures are taken in the tests to prevent such secondary effects, and to ensure that there is no carelessness or negligence on the part of operators.

Secondary effects on human beings

Even though it is not the task of the phytopathologist but the work of a toxicologist to determine the effect of agricultural chemicals on mammals including human beings, experience and information gained while carrying out the test may nevertheless prove to be of valuable assistance to the toxicologist. It must, however, be emphasized that observations in this connection must be precise and to the point, and any views of a misleading and speculative nature must be avoided.

It should be made a strict principle to obtain full information on the toxicity of the preparation before starting the field test, in order to be able to apply corresponding precautionary measures. After all, the experimenter shoulders full responsibility for the consequences arising out of carelessness.

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II. Methods for fixing the date of the test

The time when the field test is carried out has a very great bearing upon the reliability and accuracy of results obtained in plant protection tests. Variations in results obtained with insecticides are often due to the measures being carried out at different times. A test conducted at the wrong time may nevertheless produce very accurate results but they are not reliable. On the other hand, the application of a control at the right time attaches basic importance.

Generally, the most favourable time for applying a preparation or carrying out a control measure is governed by the so-called "weak spot" in the life-cycle of a pest or a pest population. The "weak spot" is understood to mean that stage in the development of a pest when it offers the least resistance to controls. It is not a firm factor but is in causal relationship to the behaviour of the pest (e. g. often concealed under plant parts etc., so that there is less possibility of knock-down) and to the stage-specificity of the preparation, and is in this case specific to the preparation. Very often narrow limits are set to the time when a test may be carried out, i. e. a control only gives the best possible effect when carried out in a certain period of time. It is essential that this period is established early and accurately, as based on the adoption of certain methods applied today on a large-scale in the so-called "alarm service", an organisation set up to warn farmers and growers of approaching pest attacks. These methods are based on

A. the results of biological research, namely

1. the knowledge of the biology of the pests and the pathogens,
2. the influence of environmental factors on the development of the pests and the pathogens,
3. the susceptibility of plants in their different stages of development, to pest infestation and infection by diseases;

B. the observation of pest occurrence by
   1. establishing the first occurrence of pests,
   2. establishing the main occurrence of pests,
   3. checking and determining the critical population density of pests;

C. the comparisons with and relations to other natural processes by
   1. determining certain meteorological conditions,
   2. observing phenological occurrences (1).

The methods are of course only useful provided the values they give are representative of the test field.

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1. Examination of plants for pest infestation

Regular examinations of the plants for pest infestation constitute a reliable method for fixing the dates of control. In fact, this method is employed with great success for a number of plant pests. The aim of this practice is to fix the control date according to either a minimum population density (critical value) or the main occurrence of pests. The general procedure adopted is to plot the values obtained from the examination of the plants in a coordinate scheme, with the time factor entered on the abscissa of the graph, and the infestation density on the ordinate.

a) Cabbage maggot *(Chortophila brassicae)*

Apart from special measures which may be taken, this pest is controlled by treating the plants with ovicidal or larvicidal preparations applied by means of the soil watering technique. In order to fix the most favourable date for the control, several of the largest plants which are primarily colonized, should be checked for the presence of eggs every three days during the period in question. By carefully bending the plants backwards and forwards, eggs laid on the root crown will be made visible. The treatment should be started as soon as the first eggs are laid.

b) Potato beetle *(Leptinotarsa decemlineata)*

The control of this pest should be started as soon as the majority of the larvae have hatched out.

c) Cherry fruit fly *(Rhagoletis cerasi)*

In order to establish when the flight of this pest commences, the old practice of examining leaf by leaf, the lower branches of cherry trees is still employed occasionally even today. The flies on the leaves are caught by placing glass tubes over them. The branches should then be shaken vigorously to see whether any flies are still present. The best time to make the check is between 10 and 11 a. m. because then the flies are certain to be present, feeding and laying eggs. This method, however, has nowadays been replaced by more reliable practices.

d) Green peach aphid *(Myzodes persicae)* and other aphid species

The 100 leaf method is employed to make checks on the colonization of potato crops by green peach aphids. The date for controlling this pest is determined by counting the number of aphids per 100 leaves. The procedure for doing this is as follows: One leaf is plucked from every 100 potato plants from one side of the field to the other, leaving out the rows on the periphery; the plucked leaves are then examined for aphid infestation. Furthermore, the leaves must be taken alternately from the top, centre and bottom of the plants so that the density of infestation will be registered for 33 lower, 33 centre, and 34 upper leaves. These leaves should be placed in a container, examined as quickly as possible with the aid of a binocular lens or a good magnifier, and evaluated numerically. Non-infested leaves can be thrown away in the field immediately. In evaluating the infested leaves, a record is kept of the number of parasitizing individuals, and other species, such as *Macrosiphum solanifolii, Doralis frangulae,* etc., which occur in addition to the green peach aphid. Particularly in the case of the green peach aphid, a distinction is also made between wingless forms, winged forms, nymphs and larvae, so that the registration of infestation will appear in the record book as follows:

	Myzus persicae				*Doralis*	*Macrosiphum*	Parasitized
Larvae	Wingless	Winged	Nymphs	Total		Total	

An exact registration of the values should also include a description of the points at which the counts are taken, comprising of such details as the location of the field, date of planting, variety, date of emergence, fertilization, commencement of blossom period (when 30 out of 100 plants flower), details on the immediate vicinity with special reference to growth of peach trees.

For virus tests, Broadbent (1) is of the opinion that it is more advisable to record the numbers of aphids per plant rather than the numbers of aphids per 100 leaves. The 100 leaf method gives greatly varying values for different potato varieties, and these differences are eliminated when the numbers of aphids per plant are counted. He, therefore, recommends the following procedure: One leaf is taken from the upper, centre and lower sections — whereby small, medium-sized and large leaves should be selected proportionately — on each of 40 plants growing at scattered points across the field. The aphid numbers counted should then be recorded separately for the upper, centre and lower leaves respectively. The average population density per leaf in the different sections of the plant is then calculated from these numbers, and the obtained values then multiplied according to the number of leaves growing in the corresponding section. The sum of these three products is equal to the infestation density per plant. For example, a count of 20 leaves would give the following rate of infestation:

top leaves	infested by 20 aphids = 0·5 per leaf
centre leaves	infested by 80 aphids = 2·0 per leaf
bottom leaves	infested by 100 aphids = 2·5 per leaf

In order to establish the infestation per plant, the average number must be determined.

By means of this method, exact tests can be conducted to determine the infestation densities on different varieties and in different locations. It should, however, be pointed out that a clear relationship between population density and spread of virus does not exist so that the numbers of aphids recorded do not on their own constitute a standard for assessing the danger which threatens the crops. The standard values occasionally given for certain regions cannot be applied generally. An infestation of less than 20 aphids per 100 leaves is generally interpreted as being slight, whereas an infestation rate of more than 100 aphids per 100 leaves is regarded as serious. As a rule, it is recommended to carry out spraying when more than 10 and 15 green peach aphids are counted per 100 leaves.

Healthy potato districts seldom have an infestation rate of more than 20 aphids per 100 leaves.

The 100 leaf method is also used for fixing the dates on which to control aphids acting as vectors of virus in beet fields (yellows, *M. persicae, D. fabae).*

As a rule, two spray applications are needed, and should be carried out at the following times:

First spray when the majority of aphids have left their winter hosts (90 % for *Evonymus* and *Viburnum)* and start to form colonies.

Second spray when new colonization is detected. For both sprays, the critical figure is one aphid per plant.

Kunze (3) has made a close study of the 100 leaf method. On the basis of extensive data obtained from investigations, he proved that the population density in a uniform field with similar ecological conditions prevailing, differs appreciably also in adjoining plots. In fact, certain values were found to differ from others by as much as nine times, and there were no changes in differences even over an observation period lasting sixteen days.

The best time for controlling *Aphis fabae* on *Vicia faba* is at the conclusion of the primary infections, i. e. when the migratory flight from the winter host has ended (2).

A proposal has been made in recent years by Shands, Simpson and Reed (4) to reduce the work entailed when sampling to determine the population density of aphids.

Church and Stickland (5) have evolved a three leaf method to determine the population density of *Brevicoryne brassicae* on brussels sprouts. They established that the difference between an aphid population actually present and that estimated amounts as a rule to between 50 and 60 %. The method they have developed aimed at saving work and time is as follows: At the end of July a brussels sprout plant has approximately 30 fully grown leaves and 10 young ones. 30 fully grown leaves are taken as the standard for determining the population density of *B. brassicae.* One-tenth of the leaves present on the plant, namely one each at the base, in the centre and at the top, are sampled. It was proved that by sampling three leaves each from plants selected at random, the population density will be determined just as exactly as when four times as much material in the form of whole plants is evaluated. For example, when a three leaf sample of fifty plants in one field is evaluated, then the use of the grid method only takes $1^1/_2$ hours as opposed to four hours for the sampling of 25 plants. It was found in these studies that with respect to

overwintering aphids, there is a greater difference between plants of different fields than between those growing in one field.

Stickland (14) has made a most interesting suggestion for reducing the work of counting when determining the population density of *Brevicoryne brassicae*. He recommends the use of an aphid counting grid whereby only one-sixth of the total number of aphids present is counted.

Banks (15) recommends a five-division rating for estimating the population density of *Aphis fabae* in the field, and a volumetric method for counting large numbers of aphids.

e) Codling moth *(Cydia pomonella)* and red plum maggot *(Grapholitha funebrana)*

The most reliable method for fixing the dates on which to control codling moth is to keep a close check on oviposition (6). The emergence of the moth is an important indication of this. The emergence of moth can be followed in the field by means of trap belts. It is a difficult procedure to check oviposition, and requires much practice. The eggs, laid chiefly on fruits, are visible in good light conditions, especially in sunshine. It is commendable to use a magnifying glass giving 8 × magnification. A record must be kept of the numbers of eggs found on at least 500 apples. When making counts, care must be taken not to touch the fruit. Experience has shown that only about 30 % of the eggs actually present are found. Those found should be effaced so that they are not counted twice. It is important for the purpose of the control that the embryonic development fluctuates between 7 and 16 days according to the temperature. Bender established that within a three year observation period, the oviposition only varied by eight days, whereas there were differences of up to 17 days in the period of the apple bloom.

The same procedure is adopted for the red plum maggot. In July, approximately 200 fruits should be checked on the tree every three days. Spraying should be carried out as soon as oviposition has commenced, and more than 10 % of the fruits are colonized. It is, however, essential to continue the observation checks. Should more eggs be laid, a further spray application must be made after an interval of a fortnight.

f) Examinations of branch specimens in fruit farming

In order to gain a reliable picture of the probable occurrence of the most important pests on apple trees, branch specimens should be taken before breaking, and examined under a binocular lens. Use should not be made of annual shoots, it being advisable to take sections of two to five year branches, 20 cm long, with short fruit wood.

Loewel and Reich (8) conducted their studies on 1 metre of fruit wood, and estimated the density of infestation on the basis of the results obtained.

Leaf suckers and spider mites:
per metre of fruit wood up to 19 eggs = insignificant
up to 299 eggs = normal
over 300 eggs = abnormal

Aphids:
per metre of fruit wood up to 4 eggs = insignificant
5 to 14 eggs = normal
over 15 eggs = abnormal

Wildholtz, Vogel, Straub and Gesler (9), in their studies, examined 200 bud shoots (wherever possible flower buds) in different parts of the crown for infestation by insects. This method proved to be most reliable for determining the population density of such insects as aphids, leaf suckers and caterpillars which remain settled in one location. On the other hand, the results obtained for very mobile species such as the quick and active larvae of capsid bugs, differed most appreciably according to the time of day and the weather conditions, so that different methods of determining population density will have to be adopted for such insects. Useful test results may be expected when the colonization of branch samples is as follows:

Aphids	50 eggs per sample
Winter moth	10 eggs per sample
Leaf suckers	100 eggs per sample
Cherry fruit moth	10 eggs per sample
Ermine moth	2 egg deposits per sample
Bud moth	5 caterpillars per sample

g) Check on infestation following bud formation in fruit farming

The checks should be commenced — but not before the rosette stage — and also completed before the start of the blossom period. A total of 200 bud shoots, wherever possible rosettes with flower buds, should be examined on two opposite sides of the tree in the upper, centre and lower sections of the crown. Leaf suckers, winter moths and bud moths should be counted separately. In the case of the ermine moth, the different, homogeneous, primary infestation points are to be taken as a unit whereas the aphids on each shoot are registered as a colony (7). For the latter, there are three categories of infestation:

1 = 1 to 5 aphids
2 = 6 to 20 aphids
3 = more than 20 aphids

In addition to the previously described practices for examining plant hosts to determine pest infestation, this method is used in many cases to fix the control date. In the case of spider mites occurring in fruit farming, the control date is determined by the emergence of larvae from the winter eggs, while for pollen beetles the control date is fixed by counting the population in a certain number (30) of bud clusters.

h) Wheat midges

In some years, wheat midges *(Contarinia tritici* and *Sitodiplosis mosellana)* occur in large numbers. The threatened wheat fields (wind-sheltered and densely growing crops are especially threatened) should be kept under daily observation in order to fix the control date. During the daytime, a check is made to establish infestation by dividing the haulm at the level of the lower leaves with the arms outstretched. When 10 or more flying midges are observed regularly over a distance of one armlength, it is an indication that the control must be carried out at once. In the evening, observations must be concentrated on flight at the level of the ears. The control must be started as soon as possible when 2 to 3 midges per square metre are observed regularly (12).

i) Soil pests

White grubs *(Melolontha spec.)*. In forests, test diggings should be carried out with three replications to a depth of down to 30 cm on a piece of ground measuring 0·5 × 2 metres. A control is necessary when the following numbers of white grubs are found:

4 of stage I/sq.m., 2 of stage II/sq.m., 1 of stage III/sq.m.

The width of the head capsule should be measured to determine the different stages:

Stage I = 2·6 mm, II = 4·3 mm, III = 6·6 mm

Grubs of May beetles are easy to distinguish from larvae of June and July beetles. When placed on the flat hand, the larvae of the June beetle soon start to creep forward most actively, whereas the grub of the May beetle lies on its side and hardly moves from the spot (13).

2. The trap net and the tree jarring trap method

The trap net is an old-established device used for determining the abundance and the first occurrence of certain pests, and for this reason is often used for fixing the dates of controls. The trap net is used primarily to catch shy insects capable of flying or insects that easily lapse into thanatosis, and which cannot be recorded quantitatively with sufficient certainty by means of described methods of checking plants for infestation. These insects include the cabbage seedpod weevil *(Ceutorrhynchus assimilis)*, flea beetles, cicadids, bugs etc. The net is essentially a bag made of fine unbleached muslin, at least 50 to 60 cm long. It is fastened to a four-sectioned loop of 1 metre circumference. The loop is attached to a handle 1 metre long. Netting of insects should be carried out in dry weather, and the bag should be swung over the plants in wide, quick sweeps from right to left with the arm outstretched so that the bag brushes the plants, although not too gently or too high up. When the sweep is not powerful enough, many insects will not fall into the bag. The bag will also tilt over, and the catch will drop out. Furthermore, many insects colonize the lower parts of the plants, and are not caught. A certain number of single sweeps gives a netted unit catch. The larger this number, the more accurate will be the result. Mostly a unit catch consists of 30 single sweeps, although sometimes a small number will also be adequate. At all events, no less than five single sweeps should be made. The insects should be netted at different parts of the plots, working from one side of the field to the other. Netting should under no circumstances be confined to the edges of the field or the centre of the plots.

After removing bees and other species which are not of interest, the catch should be emptied into glass containers or muslin bags taking care not to lose any of the insects, and provided with a label giving details of the location, date, time of day and weather conditions.

Consideration must, however, be given to the weaknesses attached to the method. Some beetles colonize the base of the plants, and cannot be netted. Others drop to the ground as soon as persons approach the plants. Often the mature females are on the ground in order to lay eggs. Furthermore, many species take to the ground to find shelter in cold weather. It is, therefore, sometimes necessary to make an additional check of the surface soil as a supplement to the netting method.

The necessity of making a quantitative assessment of the population of the species in the different plots arises especially when the secondary effects of insecticides on the biota are to be recorded. According to Tischler (10), it has been found that as a rule 50 netted catches on one stratum of the vegetation layer correspond to the colonization of an area of 1 square metre. "A comparison of the numbers of insects obtained by netting in 50 sweeps, and by means of an ocular search of a test area measuring 1 square metre leads to somewhat comparable results. A unit catch for a multi-stratified biotope, for example a hedge or the periphery of a wood, therefore consists of 50 net sweeps in the shrub layer, 50 in the herb layer, the screening of 1 square metre of ground debris and the screening of the uppermost soil layer (10—20 cm) over an area of one square metre. Naturally, the quantitative catches on which the synecological, statistical computations are based, must all be made at the same time of year and day, and under like weather conditions."

The tree jarring trap method is essentially identical with the trap net method described above. It is employed wherever the trap net method cannot be used, especially for determining rates of pest infestation on trees. The procedure for this method is to spread a sheet under the trees. Then the tree trunks or branches are jarred by striking them powerfully with a rubber-lined wooden club, in order to dislodge the insects which are collected on falling into the sheet. By this means it is possible to obtain information on the start, the peak and the end of migrations. The method must be applied with great care in order to avoid causing damage to the trees, such as scratches or bruises. This method gives reliable counts of the apple blossom weevil *(Anthonomus pomorum)*, for example. Kaiser (11) gives the following critical values for this pest. Serious infestation is to be expected when a lower branch of a 30 to 40 year old apple standard is colonized by 200 to 300 weevils. For a high bush tree, 50 to 100 weevils dislodged per jarring operation indicate a serious infestation. When there are fewer than 20 to 25 weevils per catch, it is not necessary to start controls. Generally, the values given for the netted catches apply to the number of tree jarrings to be carried out.

Instead of a sheet, use can also be made of a device resembling in its design an ordinary umbrella. This device should then be held with its hollow section facing upwards, under the branches of a tree or bush, and the branches should then be struck with the club by means of powerful, short blows in the direction of the umbrella-type device.

3. Catching insects by means of traps

Traps are finding ever widening use as a means to establish the right dates for carrying out controls of fungal diseases and insects. By means of trap devices, fungus spores and insects are caught and collected, so that information is provided on the commencement of infestation and the rate of infection.

A distinction is made between two types of traps, namely those with a purely mechanical action, and others which have an additional attractive effect.

Trap devices with a purely mechanical action include suction and sticky traps.

a) Suction traps

The literature contains descriptions of numerous types of suction traps used for trapping aphids and other flying or wind-carried insects. These traps

work on the principle of a vacuum cleaner in that insects flying past the opening of the trap are drawn in by a suction device and collected in a container. It is required of such established suction traps like those described, inter alia, by Johnson and Taylor (16) and Davis and Landis (17), that they draw in an adequate and constant quantity of air in a given period of time, and that their trapping capacity is known to within a sufficient degree of accuracy.

Bömeke (18) has described a so-called spore pump for trapping ascospores of *Venturia inaequalis*. This is a spore trap provided with a funnel, and consisting of an air-tight case containing a cover glass coated with vaseline. The funnel is mounted on a box containing blotchy leaves from the previous year. The spores are drawn in through the funnel on to the cover glass, and can then be counted by using a magnification × 250.

Hirst (19) describes an automatic spore trap used for determining the spore content of air. It operates on a similar principle to the spore pump designed by Bömeke, in that the trapped spores are also collected on a cover glass coated with vaseline.

b) Sticky traps

Small insects that fly or are carried by wind often are caught by a simple trap made up of a board coated with a sticky material. The slide method for establishing the ascospore flight of *Venturia inaequalis* works on the same lines. This method which is so important for the control of scab, is based on the following procedure. When leaf fall begins in Autumn, infested leaves are collected from varieties highly susceptible to scab, and kept at suitable places in the field, separated in batches according to varieties. They are stored for this purpose in shallow boxes covered with wire gauze. The boxes should be raised above the ground, and their bases should be perforated to allow the water to run off. The collected leaves are placed in the boxes with their upper surface to the top because the perithecia form primarly on the leaf surface facing the light. Furthermore, the leaves are exposed to all weather influences. In order to make a daily observation of the commencement of perithecium maturity, three perithecium-containing leaves of each apple variety are taken in on the previous evening. Perithecia which are almost mature, and which normally would only release the spores at a slow rate and after an extended period, then disperse the spores more vigorously next morning. If the leaves are very wet in the evening, they must be dried briefly with filter paper. They are then placed on moist filter paper in dishes, with the perithecium-containing side facing upwards. If, on the other hand, these leaves are brittle, they should be carefully wetted on the lower surface, and during the night they will become fairly supple. It is, however, essential that the adaxial surface of the leaves remains dry during this period, otherwise the spores will be discharged prematurely. Next morning, the leaves should be immersed briefly in water — each one separately — and then placed on wet filter paper in moist chambers. Fine-meshed wire nets with 18 × 18 mm cutouts should be laid on the leaves. Each cutout is positioned above a section of the leaf containing abundant perithecia, this section being located by means of a magnifying glass. At this stage, the slide spore trap, coated with a thin film of vaseline, should be placed over the cutouts with the greased side facing downwards, but should not touch the leaf surface. It is advisable to mark out an 18 × 18 mm square by means of a

wax pencil on the non-coated side of the slide, to coincide with the cutout in the net and marking the location where spores are to be sought. The distance between the slide and the leaf should be no more than 2 mm. The leaf surface should not be covered with water otherwise the necks of the perithecia will not be able to open. After the leaves have been dipped in water, they must be placed quickly on the spore traps because the mature perithecia begin to disperse spores within five minutes. Dispersal generally reaches its peak after fifteen to twenty minutes, and is more or less completed after thirty to forty minutes. The spore traps only need be left on the leaves for one to two hours in order to trap the majority of the dispersed spores.

The slides are then examined under the microscope for the presence of spores. The investigations must be continued until it has been definitely established when the spore dispersal generally begins.

In orchards, the spore traps are set up in the same manner. By observing the overwintering form of the scab fungus under the microscope, the time of perithecium maturity and spore dispersal can be established. An initial infection may be expected from this time onwards. Whether it actually occurs, however, depends upon the moisture on the leaf and upon the temperature. At high temperatures, moisture for a few hours only is needed for an infection to occur, whereas at low temperatures the moisture must persist for a correspondingly longer period. The dependence of scab infection upon temperature and humidity is indicated in the following table (approximate values).

Temperature in degrees centigrade	Infection slight hours	moderate hours	severe hours
25	11	14	21
17—24	9	12	18
16	9	13	20
15	10	13	21
13	11	15	22
11	12	18	26
10	14	19	29
9	15	20	30
8	19	25	38
7	22	30	45
6	25	34	51
1—5	more than 2 days		

A useful guide for the assessment of suction traps, sticky traps and tow-nets as suitable aids for fixing the dates of controls is provided by the paper of Johnson (20) who made a comparison of these three methods for the quantitative sampling of small airborne insects. Tow-nets are essentially cone-shaped bags one metre in length. The opening formed by the circular loop has a diameter of 75 cm. The bag is held in a horizontal position by means of a bamboo rod, and automatically adjusts itself to the direction of the wind, just like a weather vane. Whereas the efficiency of sticky traps and tow-nets is governed largely by the prevailing wind velocity, suction traps are practically

independent of wind velocity. Johnson, in his investigations, set out to establish the factors which influence the operation of the different trap devices, to compare the efficiency of the traps, and to examine the possibility of determining the population density with the aid of these traps. His procedure was to use all three devices simultaneously at the same location, and to measure the wind velocity. He found that the suction trap is superior to the other two devices for determining the population density of aphids, Coleoptera and Chironomida. Sticky traps gave better sampling of Diptera, Hymneoptera and Procoptera, possibly because these traps have a special power of attraction.

c) Bait traps

Bait traps are also widely used for catching insects. They involve the use of attractants with a more or less specific luring effect. Large-scale use is made of glass traps filled with a liquid to bait the insects. This method provides valuable information on the commencement and the peak of the flight of many noxious insects.

Cherry fruit fly *(Rhagoletis cerasi)*. Wiesmann (21) recommends the use of a 2 % ammonium stearate solution as a bait for trapping this insect. The ammonium stearate, a white milky substance, should be stirred into a paste, and then dissolved in warm water. 0·5 to 1 litre jars of the type normally used for preserving foodstuffs with a rounded edge, make good traps. The jars should be suspended with strong cords from the tops of the trees in such a manner that they can be lowered each day for checks, and then drawn up again. A total of four to five jars should be suspended in the crowns of cherry trees which are known to be heavily attacked regularly by maggots. The jars should be put out for the first time about the middle of May. The first catches indicate that the migratory flight from the soil has started. The first sprays should be applied 14 to 20 days later, followed by the second treatment after an interval of 10 to 14 days. It should be noted that the flight of the cherry fly may last from 60 to 68 days, and extend throughout the months of June and July. It is recommended to continue the observations over the whole of this period. The method is not successful, however, in cool weather.

Codling moth *(Cydia pomonella)*. Glass traps (jars) are occasionally hung in the trees also for checking the flight of codling moth. A start should be made after the petals have fallen. Various substances are recommended as baits. An established bait is a mixture of 200 ccs of apple juice and 800 ccs of water, to which four spoonsful of sugar are added. A solution of molasses with an addition of yeast, is also reputed to give good catches, but this bait is only effective as an attractant for a short period. Another recommended bait is a 10 % solution prepared from unrefined sugar, to which a little valeric acid (½ cc per litre) and a small dosage of nicotine sulphate (1 cc per litre) are added. 25 grammes of pine tar oil in a container suspended close to, but separate from the bait, increases the effect (22). Completely satisfactory results, however, have so far not been given by any form of bait.

The jars should be checked each morning, and a record kept of the number of moth trapped. The checks on the flight of the moth should give an exact indication of the date of the main flight which continues for several days. Spray applications must be carried out eight to ten days later.

In the U.S.A., baits have been developed which remain effective for several weeks; these are so-called solid baits (23, 24).

Grapeberry moth *(Clysia ambiguella* and *Polychrosis botrana).* These major pests of viticulture are known to have a liking for fermenting liquids. Glass traps (jars) are suspended in the vineyards at the end of April in warm regions, and about the 10th of May in cool districts. As a rule, four to eight jars are needed per hectare. The use of a relatively large number of jars will provide added information on the flight because usually distribution of the moths is not uniform. The jars should be set out in the middle of the vine rows, about level with the clusters. It is advisable to arrange the jars so that they are protected by foliage to keep out as much rain as possible, but at the same time it must be ensured that the moths have full access when attracted. Fermenting liquids such as beer, fruit juice or sour wine can be used as bait. A desertspoonful of sugar and a desertspoonful of vinegar can be added to each litre of bait liquid. In rainy weather, it is essential to renew the bait liquid frequently.

The checks should be carried out daily, if possible in the early morning. Normally, only the females caught in the traps are counted. The controls are started about five to six days after the flight has reached its peak.

Cabbage maggot *(Chortophila brassicae).* The best date for the control of this pest is also established now and again by trapping the flies with the aid of a bait liquid. The liquid is made up from a quarter litre of fruit wine, 3/4 litre of water and two desertspoonsful of sugar. The glass jars filled with bait liquid are placed in the field among the cabbage plants. The trapped insects should be collected daily. Only the males should be used for the numerical evaluations because it is hardly possible to distinguish the females from other *Chortophila* species. The eyes of the male almost meet on the vertex, and the frons is silver-white with a bright red, triangular spot. The eyes of the female are well apart.

d) Light traps

Many insects are attracted by light. This luring effect of light is also put to use in combination with traps to assist in fixing the dates of controls. Studies conducted by Frost (25, 26) have shown that ultraviolet light has an excellent effect.

Codling moth *(Cydia pomonella).* The codling moth responds readily to ultraviolet light, and can easily be trapped at the source of light. This is done by placing a glass jar as a trap over the light. The ultraviolet lamp is switched on before dusk.

Bauckmann (27) found that on warm evenings when the mean daily temperature was above 20° C, the peak of the flight was reached at about 10 p. m. Flying activity then became less, and around midnight the insects had almost completely stopped flying. When the mean daily or evening temperatures were below 18° C, only spasmodic flights were observed. The period of flying lasted for almost two months under the examined conditions.

e) Colour traps

Möricke (28) has developed a simple and inexpensive, but most reliable trap for investigating the flight of aphids, in particular green peach aphids. This trap is based on a detailed study of the colour vision of green peach

aphids. He uses tin dishes, 22 centimetres in diameter, with a yellow-coated base. The dishes are filled with water or a 4 % formaldehyde solution or a 0·01 % "E 605" solution. Aphids flying over the dishes are attracted by the yellow colour, and settle on the solution. In contrast to other insects, they are then unable to escape from the water. The trap is most suitable for establishing the time and the strength of the aphid flight. The numbers of aphids caught with this trap correspond to those caught with box traps and sticky traps.

4. Checking flights with the aid of collected pupae

A most reliable method of estimating the flight time of many insect species is to collect pupae, and keep them in cages under field conditions. The emergence of the insects observed in the cages will give an indication of conditions in the test field.

Codling moth *(Cydia pomonella)*

Bands of corrugated paper are wrapped around the trunks of badly infested apple trees, and then covered on the outside with weatherproof paper to give the bands protection against rain. This practice of trapping codling moth is carried out during the Summer, about the middle of July, and takes advantage of the habit of codling moth larvae of cocooning in protected places in readiness for the dormant season. Other larvae which continue their development without passing through the dormant stage, also take shelter in the corrugated paper in order to pupate and produce moths during the same Summer. The trap bands containing the cocooned larvae are removed from the trunks in late Autumn and transferred to breeding cages where they are kept outdoors in sheltered places, or else they are left on the trunk and caged in with fine-mesh wire netting in such a manner that they are protected against attacks by birds, and enabling the flight to be observed at the same time. The latter practice should be given preference because according to Urlings (29), the development of the cocooned larvae is disturbed when they are transferred to cages, so that the moths emerge later than those which overwinter in natural conditions. In the following year, the emergence of the moth in the field (commencement, peak and termination) can be checked on the basis of what has been observed in the cages. As the first eggs are laid two or three days after the moths emerge, and the first larvae hatch out about eight to twelve days later, it is at least possible to fix beforehand the date for the first controls on the basis of the emergence of moths in the observation cages. This would mean that the initial controls should be carried out about two weeks after the flight of the moths has started (30).

Cherry fruit fly *(Rhagoletis cerasi)*

Schneider and Vogel (31) have given a detailed description of checks on pupae. This method, although complicated and lengthy, is superior to all other methods. The procedure is as follows. Trees which were heavily infested the previous year, and which are colonized by a relatively large number of pupae (20 to 50 per square metre) should be picked out in January or February. The material can be left on the spot although it is of greater advantage to collect several hundred pupae, to lay them out in batches of 20 to 50 in the turf, and to mark the spots where they are placed in a suitable manner. Ants

and other predators destroy or carry away the pupae, so that it is advisable to place them in small tubes made of fine wire gauze covered with a little dry turf material or a piece of greyish-green cloth, at the same time ensuring that the sunlight still has direct access to the soil surface. High screens or breeding cages made of wood are unsuitable. The development rate for pupae which pass through the dormant stage is governed by the ground temperature after overwintering. The zero point of development is reached at approximately 7° C whereas maximum development is at 23° C. In order to obtain information as early as possible on the development stage of the pupae and on the sum total of heat required until the flies emerge, pupal samples should be exposed to a temperature of 22° C from the end of March and the end of April until the adults emerge. In May, the development stage can often be determined from the degree of colouring. For this purpose, the front part of the puparium should be opened with a needle or a pair of fine tweezers, and a special study made of the head section. A distinction is made between the following four stages of development:

1st stage — head completely white
2nd stage — eyes red
3rd stage — head completely coloured, eyes bright metallic colour
4th stage — puparium empty, flies have emerged

The first spray application should be carried out about two weeks after the first flies have emerged from those pupae exposed most to sunshine. The number of sprays to be applied depends upon how long the spray coating remains effective, upon the interval between the first flight and the probable harvest date, and upon the duration of the flight. Flat ground which is uniformly exposed and covered by an even growth of grass, and a continuous spell of warm weather provide conditions which lead to a brief, concentrated flight whereas cold, rainy weather prolongs the flight. The means that the period of the flight is governed by the weather and ground conditions. When carrying out checks on pupae, allowance should be made for the fact that on horizontal ground and with a densely foliated cherry tree, the occurrence of the main flight may easily vary by ten days on the north and the south sides. In cherry orchards on hilly ground, differences in the occurrence of the main flight amounting to four to five weeks have been established between sun-exposed and shaded parts of the ground.

Swede midge *(Contarinia nasturtii)*

Wooden boxes, 1 metre long, 50 cm wide and 20 cm high, with a round opening cut out in one side and a glass tube fitted in it, should be set up in fields infested during the preceding year by *C. nasturtii,* and where pupae are likely to be present. The midges which emerge from the pupae under the boxes, are positively phototropic, and collect in the glass tube. Approximately 25 boxes should be set up, allowing an exact study to be made of emergence from the pupae. The occurrence of 30 midges per 100 square metres constitutes a threat to the crop (32).

Wheat midges *(Contarinia tritici, Sitodiplosis mosellana)*

By means of certain methods of soil sampling and the soil elutriating practice at several sampling points, it is possible to predict the occurrence of wheat midges, and to say

1. whether a large number of larvae leave the cocoon, and move upwards to pupate. Consequently it is known whether a large flight is to be expected;
2. when the majority of the upward migrating larvae pupate. This means that it can be predicted with a fair degree of certainty when emergence from the pupae and colonization of the wheat fields can be expected (32a).

5. Observation of phenological phenomena

The development of plant diseases and pests depends largely upon the prevailing weather and the climate. The plant in its capacity as a cheap and widely spread reagent for the weather conditions (phenology), also gives an indication of the development and the behaviour of a pest population. It is, therefore, only too obvious that observations should be made of phenological phenomena to assist in fixing dates for the control of the pests. This is particularly applicable in cases where the habits of a pest are closely linked with certain stages in the development of the plant host. The spraying schedules in fruit farming are now based on phenological phenomena. The date for carrying out control measures is fixed according to development stages of the fruit trees which can easily be established and exactly defined (33, 34). It is not only the phenological phenomena of the plant host but also those of other plants, which are taken as the criterion for the development and the behaviour of pests, and evaluated for fixing the dates of controls. However, the development of the pests and the development of the plants are not completely synchronized.

Frit fly *(Oscinis frit)*

The commencement of the blossom period of geans and dandelions provided the best phenological data for indicating the occurrence of the first adults (35).

Green peach aphid *(Myzodes persicae)*

According to Rönnebeck (36) the start of the main flight coincides with the heading of rye.

Only rough indications are available for fixing the dates of controls against most fungal diseases. These dates are determined phenologically in all cases, a practice based on general experience and with a high degree of probability in its favour.

6. Determination of certain meteorological conditions

Considering that the weather conditions, in particular the temperature and the distribution and amount of rainfall, have very great bearing upon the incidence of fungal diseases, and of course also upon the occurrence of insects, methods have been adopted to forecast the dates when infections are likely to threaten and to fix the dates for controls on the basis of meteorological data. These methods of prediction are of very great importance in the case of mycoses because the aim of the controls is to afford protection against infection (prophylaxis), and infections which have already taken place can at the most be halted in the initial stage of development.

Peronospora in viticulture *(Plasmopara viticola)*

The control date is fixed with the aid of the so-called incubation calendar. By observing the weather conditions, this calendar enables the date of the probable incidence of peronospora to be forecast. It is based on the results of studies on the biology of peronospora, and the finding that in Central Europe the peronospora fungus — assuming the weather conditions to be normal — has the following incubation periods:

Month	on the leaves	on the inflorescence and young berries
Middle of May	15 to 18 days	—
End of May	12 to 15 days	12 to 14 days
Beginning of June	12 to 14 days	12 to 14 days
Middle of June	9 to 10 days	9 to 11 days
End of June	6 to 7 days	10 to 12 days
July / August	5 to 6 days	12 to 14 days

The dates can be read off exactly in a detailed calendar which includes each day. Example: When there is heavy rainfall on May 15, which may have brought about an initial infection, it can be expected that following a period of normal weather and further rainfall at the end of May, there will be an outbreak of peronospora from May 29 to June 1. This means that the vines must be sprayed before May 29.

Late blight of potatoes *(Phytophthora infestans)*

Dutch investigations on the usefulness of meteorological data for giving warnings to farmers of late blight incidence, revealed that this disease mostly breaks out after there has been a mean relative humidity of at least 82 % on two consecutive days (mean calculated from the values registered at 8 a. m., 2 p. m. and 7 p. m.), whereby on at least one of these two days the minimum temperature is 10° C or more. These conditions were established on the basis of numerous comparisons of values obtained at heights of 3·2 metres and 0·4 metres. Further studies have shown that late blight also breaks out when the mean relative humidity drops to 79 % on one of these two days. The advantage of this method is that all data given in weather charts or issued by meteorological stations can be used.

Sometimes it is possible to forecast a period in which these temperature and relative humidity conditions prevail. In this case, one or two more days are available for carrying out the sprays before the disease breaks out. The significance of this knowledge is evident from the fact that in many instances the critical period for the outbreak of disease follows several days of rainfall, during which time spraying cannot be carried out (37).

Apple scab *(Venturia inaequalis)*

The method involving the temperature sum rule dates back to a suggestion of Holz. On the basis of observations extending over a period of seven years,

it was proved that in Stade a temperature sum of daily means — calculated from readings normally taken in meteorology according to the formula

$$\frac{I + II + 2 \times III}{4}$$

— amounting to 105 ° C is necessary to render the perithecia mature for dispersion. No allowance is made in this calculation for the minus temperatures. By adding the mean daily temperatures from March 1 onwards, the date for the first spray application can be fixed.

It has been found that the temperature sums vary for the different fruit farming regions. This is associated with the varying frequency of rainfall during the time in question and possibly in the period before March 1. As the accelerating influence of alternating humidity and dryness upon the development of perithecia has been proved, it may be assumed that in a very dry region the perithecia do not develop so quickly as in a moist region, despite similar temperature conditions. A further reason for varying temperature sums in different regions may probably be attributed to the date on which addition is commenced. The perithecia are not only present on March 1 at different locations in varying stages of development, but it may also be possible that at one and the same location the development of the perithecia up to March 1 varies in consecutive years. The temperature sum in Berlin, for example, amounted to approximately 140 ° C. Therefore, the temperature sum must be determined for each region (38).

Insects

The temperature factor is also used to supplement other methods for fixing the dates of controls. The pollen beetle, for example, flies at temperatures above 15 ° C, and the codling moth, too, is only active at temperatures above 15 ° C at dusk.

7. Combined practices

By combining different methods, the dates of controls can be fixed more accurately in that the weaknesses attached to each method are offset.

Codling moth *(Cydia pomonella)*

Combination of trap jars, checks on pupae, checks on oviposition, light traps.

Cherry fruit fly *(Rhagoletis cerasi)*

Check on flight with the aid of pupae, bait trap method.

Apple scab *(Venturia inaequalis)*

Spore traps — maturity of perithecia.

Aphids

100 leaf method — colour traps.

May beetles *(Melolontha spec.)*

The biological study of the May beetle flight as the basis of the control, comprises several checks:

1. Flight samplings in the evening.
2. Check on the sex ratio.

3. Check on the stage of maturity of the females.
4. Studies of beetles collected after the treatment.
5. As a supplement: diggings in white grub meadows (beetles, eggs) (39).

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III. Methods for evaluating the test

The problem attached to the evaluation of the test is to collect material which will permit the assessment of an exact and reliable degree of efficacy. The settlement of this problem is governed by the following points:

1. the selection of the right standard for the effect of a preparation,
2. the application of this standard at the right time,
3. an adequate number of assessed samples.

Emphasis must be laid on the importance of selecting a satisfactory standard. It should be decided from case to case which standard is most suitable. But generally the same applies here, too, as in the fixing of control dates, namely that an evaluation made according to several methods, the values of which are placed together and closely compared, permits the most reliable conclusion to be drawn.

The application of the most suitable standard at the right time is just as important as the selection of this standard. The date of the test evaluation depends, on the one hand, upon the rate of action and the residual effect of a product, and, on the other hand, upon the time when the standard is most apparent and can be registered best; for example, in trial controls of the apple blossom weevil *(Anthonomus pomorum)* towards the end of the blossom when the reddish-brown, dried up flowers are clearly perceptible. The date of evaluation is of particular importance with respect to the reliability of the results. An evaluation carried out too early, just like one made too late, gives an unreliable picture.

Considering that it is only possible in few cases to establish the effect of a product upon pest occurrence on all threatened plant parts in a plot, samples are taken, and a certain number of plants or parts of plants are examined on the basis of the applicable standard. The problem of this procedure is to take samples in the evaluation of the test, which are representative of the effect of the product in the whole plot. The reliability of a single sample is very slight; in fact the reliability and accuracy of the test result increase proportional to the number of evaluated samples. The technique of sampling is also most important, and has great bearing upon the above-mentioned accuracy and reliability of the test result. It is by no means immaterial where and how the sampling is done, especially when the infestation and the distribution of the product are not uniform. The task is, therefore, to take samples which represent the average of the plots. This is done all the more successfully when samples are taken in many parts of the plots, and also from all plant parts which are of interest. It is important that the samples are taken at random. To guarantee this, the areas to be evaluated, for example in the case of agricultural crops, should be marked off with a lightly constructed wooden frame measuring 75×150 centimetres.

Finally, the samples must be taken in all plots on the same day, as far as this is possible. This applies particularly to tests against such species which have greatly fluctuating population densities.

For technical reasons, it is desirable to work with as small a number of samples as possible, which is quite understandable. This raises the question, "What is the minimum number of samples that must be taken for the evaluation of the test?" Very many studies have been conducted dealing with this question but so far it has not been possible to specify a definite number. On

principle, however, it may be said that this number can be reduced proportional to the uniformity and density of the infestation and the distribution of the product (1, 2).

The methods for evaluating the test can be divided into two groups, according to whether the pathogen or pest or the disease symptom is taken as the basis:

1. Establishing the number of dead pests and those which have survived.
2. Establishing the number of infested plants and parts of plants.

Occasionally the yield is also assessed, although mostly only as a supplement to the other methods.

1. Establishing the number of dead pests and those which survive the treatment

The number of pests which survive the application and those killed can either be counted or estimated. Whenever possible, a count should be made of insects still alive on the plants as well as dead ones still present on the plants or on the ground. It is, however, only possible to count large insects on the ground, and also when the soil is not covered with vegetation. Furthermore, counts of insects on the ground are rendered difficult due to the insects hiding under the top soil or in cracks so that they cannot be recorded. Insects affected by poison often become restless and leave their host plants. For this reason, it is sometimes impossible to make a complete count of the dead or injured insects when preparations with a slow action are used so that only a count or estimate of the unaffected insects still present on the plants can be made.

The check on the success of the application must of course be carried out on the same day for all preparations used and in all plots. This is necessary especially in the case of polyvoltine species because their population density often changes most appreciably within a very short space of time so that an evaluation carried out at different times gives results that are absolutely and relatively incorrect. The necessity of having to evaluate all test plots at least in the course of one day often involves a great amount of work so that the extent of the actual test work is considerably restricted. For this reason, methods which enable the evaluation of the test to be accelerated, are of the greatest value.

Spider mites *(Tetranychidae)*

The population density on a certain number of leaves provides the standard for the effect of a control measure. Unterstenhöfer (1) has carried out a close study on the technique of evaluating spider mite tests. The following recommendation is made. For determining the population density, between fifty and one hundred leaves should be evaluated. The deviations are greater at the beginning of the occurrence than during the further course of vegetation. Should fine differences be established among different preparations, then a larger number of leaves must be taken. The evaluation must be carried out on the same varieties and trees growing under the same local conditions. In addition to the ocular evaluation with a magnifier, several rapid and reliable recording methods have been evolved. Austin and Massee (2) developed the imprint recording method. In this method, the leaves to be examined are placed between two sheets of white paper, and passed through a hand wringer. Eggs and mites are squashed in the process, and leave characteristic and indentifiable spots on the paper. By means of this method, it is possible to take almost

any number of leaf samples from many plots in one day and to make the count without haste. Chapman and Lienk (3) refer to a method by which the leaves are gasified in vessels with ethylene dichloride, and the dead mites are then removed by means of a special brush device and counted. Newell (4) suggests a method in which the mites are washed off the leaves by means of a soap solution, and then counted.

With the aid of a plan drawn up on the basis of careful studies on the period of the different stages of development in relation to the temperature, certain information can be obtained on the stage-specific effect of acaricides. For this purpose, an ocular evaluation of the test is necessary, discriminated according to stages (1).

Just as with spider mites, the same procedure is also adopted in respect of other pests for which the effectiveness of a preparation is determined on the basis of the population density on a given number of plants or parts of plants. As a rule, the same values given in the section dealing with the fixing of control dates, apply here to the number of samples required. Use is also made of the same methods for determining population density, e. g. ocular examination of plants, trap nets, tree jarring trap method with sheet spread out below the tree to catch insects dislodged by jarring the trunk or branches with a club, and the different types of traps such as light, sticky and bait traps.

Codling moth *(Cydia pomonella)*

Richardson (5) has dealt fully with the evaluation of codling moth tests. The usual method of determining the infestation of codling moth is to establish the extent of injuries on fruit picked in Autumn. In this method, the evaluation unit is a tree from which 50 to 300 apples are selected at random and examined. It is important also to include fallen fruit because apples attacked by the codling moth mature earlier and drop off the tree. All fruits which have entry or emergence holes bored in them must be cut through to make sure that the damage has actually been caused by codling moth because the injury inflicted by these worms may easily be mistaken at first appearance for that caused by other fruit pests.

This method, however, does not give any indication of increase in infestation during the vegetation season. In order to obtain this information, Richardson suggests the following procedure:

Sampling should be carried out on the lower peripheral parts of the tree; the examined fruits should not be plucked. It is a known fact that the fruits in the top of the tree are more heavily attacked than those growing in the lower parts. But the infestation in these lower parts only fluctuates slightly. This also applies to control measures in which the lower branches are more uniformly sprayed, and where the spray coating is less exposed to rainfall. When the fruits are left on the tree, the evaluation can be repeated several times. In doubtful cases, a magnifying glass should be used, or the fruits should be cut through. The best criterion is "infested fruits per unit of examined fruits". It has also been established that there is a correlation between the density of hanging fruit and the population density. The greater the density of hanging fruit the smaller will be the relative infestation, and vice-versa.

The same procedure is also adopted for the red plum maggot *(Grapholitha funebrana)* as that used for the codling moth.

Sometimes it is not possible to evaluate field tests on the spot with adequade accuracy. In such cases, the evaluation should be carried out in the laboratory or in the greenhouse. This is customary, for example, when testing ovicidally effective winter sprays, and also for testing the residual effect of insecticides. Generally speaking, however, it is recommended to carry out a so-called field-laboratory test wherever the opportunity presents itself, because on the one hand this gives more accurate quantitative values, and on the other hand additional experience can be gained particularly regarding the distribution of the preparation over the different parts of the plants. For this purpose, plant parts infested by parasites which have definitely perished under influence of the poisons, are sampled and the mortality is established. Another alternative is to sample non-infested plant parts only, and to colonize them artificially with pests, occasionally making use of species which have been bred in the laboratory. It must, however, be borne in mind that this method of evaluation does not necessarily provide information on processes in the field because on the one hand the conditions for the toxic effect are different, and on the other hand the insects are unable to migrate, in other words caged-in insects cannot leave the poisonous zone.

For testing winter sprays against eggs of apple suckers *(Psylla mali)*, pieces of branches are taken into the laboratory, and marked off with rings of glue before the larvae emerge. The tests should be evaluated as soon as all the eggs on the check branches have hatched out. In order to make a better examination, the branches should be cut into sections, and a count taken of the dead eggs, taking care not to mistake these for unfertilized eggs. Eggs which have hatched out may be recognized from the narrow longitudinal slit; unfertilized eggs are white and considerably contracted, whereas fertilized eggs are dark-brown and mostly still taut. At the same time, the larvae present in the leaf and flower clusters should be counted.

The same procedure is followed for aphids. It should, however, be noted in the case of these pests that the percentages of hatchings vary greatly from year to year, from tree to tree in one year, and even from branch to branch in one tree. Between 10 % and 70 % of the eggs may be unfertilized. Differences of between 30 % and 60 % are to be regarded as normal.

The evaluation of trial controls against the cherry fruit fly is also carried out in the laboratory. Use is made of the salt water test; 350 grammes of common salt are dissolved in one litre of water. The stalks should be removed from a counted number of cherry samples, mostly 25 to 50. These samples should then be crushed between the thumb, forefinger and middle finger under the surface of the liquid. The content of the glass should then be kneaded thoroughly, care being taken to avoid the formation of foam. The maggots that are present can then be counted a few minutes later.

2. Determination of infested plants or parts of plants

The injury caused to the plant is often taken as a standard for evaluating a test, especially in fungicidal and virological tests. It must, however, be remembered that the disease symptom is not always specific, so that it cannot be concluded from the injury that this has been inflicted by a particular pest unless proved beyond all doubt. Nevertheless, it is possible in the case of a number of insects (mining pests, galls, etc.) and fungal diseases to infer from the injury with a fair degree of certainty which causal organism is responsible

for the injury. It is, therefore, of the utmost importance to have an exact knowledge of the disease symptoms.

Generally, ratings are used in addition to counting and estimation methods — the latter in particular — when the injury is taken as the standard. This means that ratings are very important.

Peronospora *(Plasmopara viticola)*

200 leaves of equal age are examined, and the surface area covered by peronospora is estimated according to the following rating:

0 = no infection	
1 = very weak infection	(= up to 1/8 of leaf surface white)
2 = weak infection	(= up to 1/6 of leaf surface white)
3 = moderate infection	(= up to 1/4 of leaf surface white)
4 = serious infection	(= up to 1/2 of leaf surface white)
5 = very serious infection	(= more than 1/2 of leaf surface white)

By adding the values for the different leaves, a total value is obtained for each preparation, which is compared with the untreated check and with the infection on the vines sprayed with comparison preparations.

Another evaluation rating is based on the effect, and not on infection. The effect is assessed according to appearance, and rated as follows:

1 = effect very good
2 = effect good
3 = satisfactory
4 = not satisfactory
5 = completely unsatisfactory

Furthermore, degree of infection is also assessed according to appearance of berries attacked by peronospora:

0 = no infection
1 = very weak infection (isolated up to 5 %)
2 = weak infection (uniform up to 10 %)
3 = moderate infection (uniform up to 25 %)
4 = serious infection (up to 50 %)
5 = very serious infection (more than 50 %)

The same system is followed for mildew and other peronospora diseases.

Apple scab *(Venturia inaequalis)*

The following rating is recommended for this disease:

weak infection	= 1 to 6 small spots (of less than 5 mm in diameter)
moderate infection	= more than 6 small spots or 1 spot > 6 mm
serious infection	= fruit covered all round with spots or has isolated large spots so that the fruit becomes stunted

Croxall, Gwynne and Jenkins (6) recommend the following rating:

The assessment is carried out on 10 trees per variety, distributed evenly in the plot. Five branches are taken as a sample unit from each tree (from different parts around the tree). Each tree is assessed separately, and the mean is then computed.

Scab %

0·01 Occasionally spots are present on 1 or 2 fruits in a sample.

0·1 Small spots on 1 or 2 fruits of every sample.

0·5 Small spots on approximately a quarter of the fruits in every sample.

1·0 Up to 5 small spots on approximately half of the fruits, no fissures.

5·0 Spots on approximately 75 % of the fruits, fissures occasionally.

10·0 Infection on each fruit, with several fissures.

25·0 Serious infection on each fruit, with cracks on 25 % of the fruits. Difference in size of fruits, on the whole somewhat smaller than healthy fruits.

50·0 Large spots on each fruit, with cracks in one third of the fruits, not fit for sale, fruits smaller than those from healthy trees.

75·0 Large spots on each fruit with cracks in half of the fruits, not fit for sale, fruits appreciably smaller than those from healthy trees.

The same system is followed for leaf scab. During the vegetation period, 500 leaves (50 each from 10 trees) are sampled to determine serious, moderate and weak infection.

Generally, considerable difficulties are encountered in the assessment of leaf diseases, whereas diseases of fruits can be recorded with greater accuracy.

On the basis of a detailed study of the different factors which have to be considered in the evaluation of scab tests, S o e n e n (12) recommended the following methods of assessment. They are based on two possibilities of infection by apple scab, namely the primary infections by ascospores and the secondary infections by conidia *(Fusicladium dendriticum)*.

The assessment must be started as soon as the first spots are visible, and continued according to the life-cycle of the parasites. Consideration must be given to both leaf and fruit scab. To save work and to increase the degree of accuracy, use is made of a counter. The following rating is recommended for leaf scab:

0 = leaf not infected
1 = leaf showing one spot
2 = leaf with 2 to 5 spots
3 = leaf with 6 to 10 spots
4 = leaf with 11 to 20 spots
5 = leaf with more than 20 spots

For fruit scab

0 = fruit not infected
1 = fruit with several spots
2 = fruit blotchy all over

The number of leaves or fruits to be examined depends upon the degree of scab incidence.

Severe incidence	50 leaves
Moderate incidence	100 leaves
Slight incidence	750 leaves
Moderate incidence	100 fruits

Late blight of potatoes *(Phytophthora infestans)*

Large (7) recommended the following ratings for estimating late blight of potatoes in the field:

Late blight in %	
0·0	No late blight perceptible.
0·1	Odd plants infected; up to 1 or 2 spots in a radius of about 10 m.
1·0	Up to 10 spots per plant, or slight covering of spots generally.
5·0	Approx. 50 spots per plant, or a spot on about every tenth leaf.
25·0	Lesions on practically every leaf, plants still growing normally, field smells of blight but still appears green although each plant is infected.
50·0	Each plant infected, and approx. half of leaf green destroyed. Spotted green appearance of field with a brownish hue.
75·0	Approx. 75 % of leaf green destroyed. Field appears neither predominantly green nor brown.
95·0	Very few green leaves, stalks still green.

Bean aphid *(Doralis fabae)* on *Vicia faba:*

Müller (8) used the following rating method for assessing the aphid population density:

0	No infestation.
i	Initial infestation, and small colonies of females with or without breed, which are not visible unless a close inspection of the plants is made.
I	Terminal shoot so densely colonized by aphids that it appears "black" even at a glance.
II	In addition to the terminal shoot, lateral shoots are also completely covered with aphids, in particular the bud and flower shoots; the main stem, however, is still free from infestation.
III	The main stem, too, is densely colonized by aphids below the terminal, down to at least the third layer of leaves. Plant shows signs of becoming affected.
IV	The whole of the main stem is black with aphids almost down to the ground. Plant seriously affected.
V	Indicated by IV when the plant is deserted due to migration of winged aphids. Black covering is full of gaps, but the numerous exuviae (skins) indicate how dense the infestation has been. Mostly the plant is very seriously injured.
VI	Like V, but terminal is bent over. Plant is limp but still more or less green.
VII	Plant completely blackish-brown, withered or collapsed.

Sedlag (9) used the following ratings for assessing the population density of aphids:

Rating	No. of aphids
1	1
2	2—5
3	6—20
4	21—100
5	> 100

Yellows of beets

The incidence of yellows can be assessed on the basis of the infection ratings given by Dame and Goossen (10):

Serious infection: 100 % incidence. All areas infected early (July/August). *M. persicae* is mainly responsible for the spread of infection. Average yield losses of 20—30 % and 15—20 % for beets and tops, respectively. 1 % drop in sugar content, up to 50 % reduction of nutritive substance in leaves.

Moderate infection: Incidence up to beginning of August. 50 to 60 % infection, by middle of September 100 % infection. Appreciable damage chiefly in fields set late and where the crops are full of gaps.
Average yield losses of 10 to 20 % and 10 % for beets and tops respectively, 0·5 % drop in sugar content, up to 30 % reduction of nutritive substance in leaves.

Slight infection: Late incidence. Transmitted mainly by *D. fabae*, and later by *M. persicae* if at all. Up to 70 % infection in conditions favourable for diseases to be transmitted.
Average yield losses: serious losses are mostly local. The decrease in the value of tops following late infection may, however, be more serious than in regions hit by earlier and serious infections.

Cabbage maggot *(Chorthophila brassicae)*

When the crop is harvested, the number of marketable cabbage heads and the maggot injuries on the roots of the removed cabbage stalks are determined, e. g.:

	% marketable heads	% infested or damaged roots
Preparation x	94	8

Examination of virus-infected plants

a) Serological assay.

b) Physicochemical assay
e. g. Goldsol reaction, turbidity measurement, staining.

c) Assay on test plants
e. g. *Capsicum annuum, Solanum demissum, Datura stramonium, Physalis angulata, Physalis floridana.*

Evaluation of the biological pest control

Franz (11) has described the methods for assessing the success of biological pest control. They may be classified as follows:

1. The mortality of pests when the activity of natural enemies is undisturbed.
2. Check on the mortality of pests when the activity of natural enemies is partially restricted (effected by mechanical, chemical and biological screening of the predators).

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IV. Assessment of effectiveness

The purpose of the field test is to provide an answer to the question as to whether and to what extent a plant protection measure or the application of a plant protection product exerts an influence upon the density of infestation or the scale of injury inflicted by diseases and pests. The test should, therefore, enable differences in infestation or injury to be registered. These differences

are obtained either by comparing treated and untreated (check) plots or by comparing treated plots and standard plots in which use is made of preparations or control methods with a known effect, making allowance for the untreated check. An exact field test, therefore, includes

untreated checks, test preparations or trial methods and, if necessary, a standard.

The purpose of the untreated check is to obtain information on the life-cycle of a pest or disease, its effect and scale of occurrence, in other words to establish the increase of infestation under normal, uninfluenced conditions. Frequently a serious infestation suddenly collapses due to nature, and often it assumes an abnormally high degree. The knowledge of the natural course of pest occurrence is indispensable for assessing the effect of a preparation or a control measure.

Once a test has been carried out, the most important task is to express directly or indirectly the effects of a preparation on a causal organism or a pest or on the infestation and the degree of infection of cultivated plants. The most exact way of expressing such effects is always to quote figures.

As a rule, the value of an insecticide or allied product is expressed by quoting its effectiveness. The effectiveness of the product indicates the percentage reduction in population density or injuries caused by diseases and pests, as brought about by a preparation or a control method in comparison with untreated plots. In other words, the decrease in infestation or the reduction of injuries is taken as the standard for assessing effectiveness, and not the yield increase factor — although the latter might appear more advisable from the aspect of economic studies — such as is mostly the case, even though not always, with variety and fertilization trials in the agricultural field test. With few exceptions, consideration cannot be given to the yield increase because a number of disturbing influences may be brought to bear on the yield, which are not related to the effect of the preparation, and consequently have no connection with the test problem, thus obscuring the effect. The yield can only be taken as a standard when it is influenced solely by the effect of the preparation. For this reason, the selection of the right standard for assessing the effectiveness is of fundamental importance, a standard which, uninfluenced by other processes, clearly reflects the effect of a preparation or a control method. Generally, the influence of the control measure upon the causal organism or upon the injury inflicted by this organism, is taken as the standard for assessing effectiveness. Whenever possible, preference is given to the former. An improved symptomatology will be of advantage to the exact determination of effectiveness in all cases where the pest itself cannot be taken as the basis of assessment.

The effectiveness can be determined numerically or by estimate on the basis of ratings or by a combination of both methods.

Various formulae are used for computing the effectiveness. The formula generally applied today is that worked out by A b b o t t (1), namely:

$$\text{Effectiveness expressed in } \% = \frac{C - T}{C} \times 100$$

$$= \frac{\text{living organisms in check} - \text{living organisms in test}}{\text{living organisms in check}} \times 100$$

This formula originally valid for laboratory tests can be reformulated on the basis of reliable test results, and used also for field trials:

$$\text{Effectiveness expressed in } \% = \frac{\text{infestation in check} - \text{infestation in test}}{\text{infestation in check}} \times 100$$

This formula makes allowance for all factors important for carrying out an exact assessment of effectiveness, in particular natural mortality in the untreated check.

The effectiveness given as one figure by this computation provides a clear and more reliable picture than ratings and other indefinite values such as "serious", "moderate" and "weak".

Occasionally, the following formula is to be found, for example in the publication of Schneider-Orelli (2):

$$\text{Effectiveness expressed in } \% = \frac{b - k}{100 - k} \times 100$$

$$\text{Effectiveness expressed in } \% = \frac{\%\text{ dead in test} - \%\text{ dead in check}}{100 - \%\text{ dead in check}} \times 100$$

This formula is merely a modification of Abbott's formula. Both give the same values. The latter, in contrast to that of Abbott, is not based on the living but on the dead organisms, in other words upon the success achieved. Accordingly, the difference between dead organisms in the test and dead organisms in the check is given in relation to the difference between 100 and dead organisms in the check (= natural mortality). This value, however, is identical with the percentage of living organisms in the check, and the first value is equal to the difference between living organisms in the test and living organisms in the check, since dead in the test minus dead in the check = living organisms in the check minus living organisms in the test.

Example of how to apply Abbott's formula:

Living organisms counted in check = 88

Living organisms counted in test = 7

$$\text{Effectiveness expressed in } \% = \frac{88 - 7}{88} \times 100 = \frac{81}{88} \times 100 = 92\%$$

Healy (3) has compiled a table including Abbott's correction for natural mortality.

Henderson and Tilton (4) refer to a modification of Abbott's formula specially intended for assessing tests carried out to control mites:

$$\%\text{ kill} = 100 \left(1 - \frac{Ta \times Cb}{Tb \times Ca}\right)$$

Tb = No. of mites before treatment

Ta = No. of mites after treatment

Cb = No. of mites in untreated check before treatment

Ca = No. of mites in untreated check after treatment

Example:

Tb = 312	Cb = 460
Ta = 26	Ca = 530

According to the above formula, this would give a kill of 92·8 %.

Before Henderson and Tilton published their above formula for calculating the effectiveness, Sun and Shepard (5) had already pointed out that by using Abbott's formula for certain objects appreciable errors may arise which limit the use of this formula. Although the formula makes allowance for natural mortality, in other words the natural decrease in infestation, by including the untreated check, it does not make allowance, or only incompletely, for the natural increase in infestation. Generally, an increase in infestation was tacitly taken to equal a wanting natural mortality. Consequently the increase in population in relation to the effectiveness was left unconsidered. In the case of insects which reproduce rapidly or which migrate to the infested zone or hatch out constantly, however, the increase in infestation may within a short period be greater than the initial population was, so that when this factor is neglected, not only the lack of an effect but also even an increase in infestation may be attributed to a preparation. An increase in population of up to more than 100 % was established within 24 hours for the cabbage aphid (*Brevicoryne brassicae*), while the increase in population registered for *Thrips tabaci* even after 72 hours was more than 800 %. As the result of migration, an increase in infestation of approximately 450 % was recorded for the pollen beetle (*Meligethes aeneus*) within five days (6). These changes in population density must automatically lead to wrong results concerning the effectiveness of a chemical, when they are disregarded. The degree of effectiveness is influenced just as much by increases in infestation as by a drop in infestation. Undoubtedly, this is a reason for some of the greatly varying data of different experimenters on the effectiveness of the same substance even when the indications are the same.

Sun and Shepard have eliminated this source of error by using the following formula for determining the effectiveness of a preparation, when the increase or decrease in infestation is known.

$$\text{Corrected percentage of population decrease (effectiveness expressed in \%)} = \frac{P_t \pm P_c k}{100 \pm P_c k} \times 100$$

P_t is the estimated or counted mortality as according to the difference between the numbers of living insects before and after application of the preparation, without the dead insects being counted, which incidentally is hardly possible in the field test, and for that matter in the laboratory test, too, in the case of aphids, spider mites etc., and if carried out it involves considerable time and large counting errors. The value P_t, therefore, represents the result given according to the mortality determination to date, which with respect to the untreated plot is not corrected, in other words the effectiveness in terms of mortality figures.

$P_c k$ represents the value for change in population, in other words for increase or decrease in infestation, being positive when the population increases and negative when it decreases. The formula, therefore, ensures that allowance is made for the initial population of the insects before the treatment, and that the effectiveness of a chemical is not expressed in values of infestation decrease

solely according to the number of insects in the untreated check, which was previously the case. Natural mortality and increased infestation occur simultaneously. The determined values for change in population, however, include both factors.

The derivation of the above formula is based on the following conception. When the insect population in the untreated check increases during the period between the application of the preparation and the evaluation of the test there will be a corresponding increase also on the treated plants if a treatment were not to be carried out. Nevertheless, due to the effect of the preparation, the increase in infestation on the treated plants will not be so great as in the check. For this reason, the actual increase in population on the treated plants is also smaller than the theoretical increase that is calculated. However, when the effect on insecticides is expressed in values of the inhibition of the population increase instead of in values of the degree of mortality, then the slighter increase in population on the treated plants will have to be attributed to the effectiveness of the preparation, because after all it is due to the effect of the insecticide.

Example 1: The same values are used as those given in the example illustrating the formula of H e n d e r s o n and T i l t o n :

	Mites per leaf sample	
	Check	Treated
At start of test	460	312
At evaluation of test	530	26
Increase of population (530—460 = 70)	in %:	15 %
Determination of mortality (312—26)	in %:	92 %

$$\text{Corrected effectiveness} = \frac{92 + 15}{100 + 15} \times 100 = 93\,\%$$

Example 2: Pollen beetle test:

	No. of beetles per 10 buds	
	Check	Treated
At start of test	20·7	20·7
after 5 days	92·7	11·0
Increase of population (92·3—20·7 = 71·6)		in %: ∼ 340 %
Determination of mortality (20·7—11·0 = 9·7)		in %: ∼ 47 %

$$\text{Corrected effectiveness} = \frac{47 + 340}{100 + 340} \times 100 = 88\,\%$$

The examples show that in the case of an increase in population, the non-corrected value is smaller than the result corrected according to the above formula.

Despite the fact that established formulae are available, many experimenters still continue even today, simply to give the established mortality figures or even only the differences in population density established in the various test rows, and frequently no allowance is made even for the values in the untreated check. It is then left to the reader whether and to what extent he agrees with the conclusions drawn by the experimenter. It is, however, misleading when no allowance is made for the untreated check in the publication of test results,

and it cannot be understood why the observations made in the check should not be included in the calculation of the values obtained in the test rows when computing the effectiveness of a preparation.

Sometimes it is not possible, or only by taking up considerable time, to express the effectiveness quantitatively and precisely on the basis of exact counts. In such cases, use is made of ratings and the estimated observations are grouped in these ratings so that the effectiveness of the preparation is expressed in terms of an estimated rating. The use of such ratings, however, is accompanied by the danger of subjective evaluation. Counts are always more objective.

Generally use is made of a five-scale rating of the following kind:

I	No injuries or	very good effect or	full or practically full effect
II	Slight injuries	good effect	high percentage effect
III	Moderate injuries	moderate effect	medium percentage effect
IV	Serious injuries	weak effect	low percentage effect
V	Very serious injuries	very weak to no effect	very low percentage effect

Certain difficulty is experienced in limiting the different scales of the rating. Bolle (7) has made the following suggestion. The entire series of 0 to 100 % should be divided by means of geometric (logarithmic) progressions, and not by equal arithmetic divisions. Each following class is q times as large whereby q may be $\leqq 1$. When the lowest class is large, the following classes should be progressively smaller. When $q = 1/2$, the following progression is obtained:

$$a + \frac{a}{2} + \frac{a}{4} + \frac{a}{8} + \frac{a}{16} = 100$$

$$a\,(16 + 8 + 4 + 2 + 1) = 100 \times 16$$

$$a = \frac{1600}{31} = 51{\cdot}613$$

$$\frac{a}{2} = 25{\cdot}806$$

$$\frac{a}{4} = 12{\cdot}903$$

$$\frac{a}{8} = 6{\cdot}452$$

$$\frac{a}{16} = 3{\cdot}226$$

The class limits, therefore, are as follows:

V = 0— 51 % mortality or 100—49 % infestation
IV = 52— 77 % mortality or 48—23 % infestation
III = 78— 90 % mortality or 22—10 % infestation
II = 91— 96 % mortality or 9— 4 % infestation
I = 97—100 % mortality or 3— 0 % infestation

It is important that q is correctly selected, which is the case when the two top classes I and II cover that tolerance which at present can be expected of a good pesticide.

The previously described formulae of Abbott, Scheider-Orelli, Henderson and Tilton, and Sun and Shepard for computing the effectiveness of an insecticide can only be applied when the test results are determined numerically. In those cases where a combination of counts and estimates is applied such as is customary especially for fungal diseases, the computation of the results to give a degree of infestation (which in principle is also the effectiveness) is carried out according to the method proposed by Townsend and Heuberger (8).

$$P = \frac{\text{sum } (n \times v) \times 100}{5\ N}$$

P = degree of infestation

n = number of leaves in each infestation category.

v = numerical values of infestation categories (ratings)

N = total number of leaves

The plant organisms to be examined are placed in 6 categories or ratings (0 to 5) according to the degree of infestation, 0 being no infestation and 5 complete destruction. This rating can be adapted as required to the objects being assessed.

The practice may be explained by quoting an example given by Wenzl (8). In one test carried out to control leaf spot of beets *(Cercospora beticola)*, approximately 80 % of the foliage was destroyed. No new growth developed. 30 leaves were evaluated, and these were grouped in the infestation categories as follows:

N = 30	n	v
	24	5
	1	4
	1	3
	1	2
	1	1
	2	0

$$S\ (n \times v) = 130\ \frac{P = 130 \times 100}{150} = 86{\cdot}7\ \%$$

Wenzl has defined the limits set to the application of this method. Following a detailed study, he arrived at the conclusion that this method of computation can only be applied when the value N is not dependent upon the extent of disease incidence. "On the other hand, in all those cases in which new leaves are formed following the destruction of the foliage due to serious incidence of *Cercospora* leaf spot disease, as on sugar beets, it may be expected that the value P shows a large or small error."

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V. Checking the exactness of the effectiveness of the preparation

Even when tests are carried out and evaluated most exactly, the results obtained in the different replications of a test series will vary somewhat. The reason for this is that the field test is a biological test, and apart from the factors being tested which are controlled by the experimenter there are also numerous influences that play a part and which cannot be controlled. Consequently, the field test never provides absolutely correct values of general validity but only approximate values which are subject to a certain degree of inaccuracy and insignificance.

In view of this, it is obvious that as part of the test evaluation, the objective check of the results is most important and in fact indispensable if the subjective assessment is to be avoided. This automatically leads to the use of mathematical-statistical methods.

Whereas the use of statistical checking methods is accepted as a matter of course in field tests conducted for the purpose of dealing with questions relating to varieties and fertilization, these methods are not used in the large majority of cases in plant protection test procedure. Primarily, the reason for this is that conditions prevailing in plant protection are completely different from those in plant cultivation, not to mention that text-books dealing with mathematical-statistical methods never give any examples relating to plant protection. Furthermore, in very many cases the differences between the test object and the check are so large that methods designed to give a statistical significance of differences appear superfluous. Nevertheless, one should not fail to recognize that it is not only necessary to establish how large the difference is between the degrees of effectiveness of two preparations or control measures but also to establish whether and to what extent these differences are significant. In principle, the same applies to the field test as to the plant cultivation test, namely that a statistical check of results is always necessary when the results are given as numerical values irrespective of whether counts, estimates or ratings are involved. It is only when no numerical values are given that

there is no possibility of using statistical check methods. When these are used, it is, however, necessary to keep in mind the special conditions prevailing in plant protection as opposed to plant cultivation. It is important here to select the yield as the standard only in rare cases, and mostly to use such standards which are directly linked with the measure being studied, e. g. number of infectors, number of destroyed or injured plants or plant parts, number or percentage of killed or survived pests, failures etc. These standards which must always be fixed from case to case, allow the differences in the effect of insecticides or crop protection measures to be established more clearly and, in particular, more correctly and instructively than the yield. This has nothing to do with the fact that the last-mentioned standards often show a relatively high mean error. Finally, it often happens that spot checks are available from other basic total values "for which the deviations are correlated or equal to the means. This condition brings about fundamental differences with respect to the statistical check, which often constitute an impediment" Wenzl (1).

Lein (2) is to be agreed with when he says "that a test procedure can only be regarded as expedient when it provides a maximum of information after exploitation of the possibilities offered. It is obvious that the statistical methods of evaluation and their reactions on test planning contribute towards improving this information. To what extent this is applicable can only be judged by the person who knows the possibilities and has examined them ... One should not go to extremes in dealing with small problems although it may prove worthwhile when use is made of precision methods. The statistician by no means has faith in figures. On the contrary, his profession is to criticize figures."

Generally, the purpose of biological statistics is to make a numerical-technical evaluation of results obtained with biological material (3). The results are expressed in figures and a comparison is made of the mathematical characteristics of various groups of material in order to establish differences and to check their conformity with certain hypotheses. In biological statistics, the available material is always understood to be a sample of an infinite statistical population, i. e. each test result is regarded as a spot check from the basic totality of all possible results. Whereas the mathematical characteristics such as means and deviations, which are computed from the samples, are merely approximate values, the corresponding values of the statistical population are exact values. We are, however, interested in the real values of the statistical population with a view to making forecasts, which is the ultimate aim. In order to determine these, limits are computed from the available sample according to certain mathematical rules; it is between these limits that the real values of the population are likely to be found. It is interesting to note that with statistical methods absolute correctness can never be proved, but that it is only possible to determine the limits between which the real values lie. The determination of the threshold values is just as significant as the determination of the degree of exactitude on the basis of which the real values are computed approximately. The main purpose of the statistics is, therefore, to determine the error of the approximate values. This is done with the aid of formulae which are valid for certain standard curves and applied to the results of the examined sample. When the results of a series of measurements are plotted in a coordinate scheme, with the characteristics on the abscissa and

the frequencies on the ordinate, a curve is obtained which gives the composition of the sample in relation to the examined property. Curves obtained in this manner have been proved by experience to resemble certain standard curves for which the exact mathematical properties are known. However, as the obtained curves do not actually conform exactly with the corresponding standard curves, a statistical treatment is always only approximate, an unavoidable fact because it is not possible to analyze each individual curve.

A standard curve of very great significance is the so-called normal curve — also referred to as the binominal curve — because the numerical values of biological material can frequently be plotted to give curves which conform largely with the normal curve or its known variants. The typical normal curve is symmetrical and has one peak, the peak value being the mean.

1. Computation of the mean value and the mean error

In each field test, it is extremely important to form a mean value from a number of single observations, because all single observations never produce exactly the same result no matter how precise the observation and measuring methods may be. These results always vary to some degree. The causes of these deviations lie in the unavoidable, small measuring and observation errors and the natural fluctuations of the objects being measured. Consequently, the most frequent value or the mean appears in place of the real value. The mean which is calculated from a sum of individual values, is an absolute figure, and it may be assumed that it is very close to the sought real value. The convincing power of a mean depends largely upon how the single values are distributed on either side of it, because the same arithmetic mean may be obtained from the most varied numerical values. The problem, therefore, lies in the fluctuation of the single results on either side of the mean. A standard is needed for this fluctuation. The best standard for accuracy is the determination of the mean error by means of the error probability calculus. Computation is carried out according to the method of least squares, based on the normal law of errors of G a u s s. The mean error of the mean value M ± m is computed according to the following formula:

$$m = \pm \sqrt{\frac{\text{sum } (x - M)^2}{n \times (n - 1)}}$$

x = single value
M = mean value
n = number of single values

To give a definite characterization of the mean error, it is necessary to quote the number of single values.

2. Determining the significance of differences in effect

As already mentioned, the plant protection test is intended to establish differences in effect in terms of differences in infestation or injury. It is extremely important to determine whether and to what extent the established differences in effect are significant. W e n z l (1) has made a thorough assessment of, and expounded statistical check procedure suitable specially for the

plant protection test. The paper of Wilcoxon (3) is of great value for the first computation check. In this paper, he describes the "rank methods" which may be applied to different problems. The great advantage of these methods is that they involve little calculation work.

A long used procedure for checking the significance of differences is to compute the Z value. The Z value is the quotient of the difference between the two means $D = M_1 - M_2$ and the mean error of this difference mD.

$$Z = \frac{D}{mD} \qquad mD = \sqrt{m_1^2 + m_2^2}$$

In test procedure, a difference used to be taken as significant when the Z value was at least 2, which corresponds to a probability of 1:19.

It has, however, been proved that confidence can only be placed in this computation when the number of replications from which the different means are calculated, is sufficiently large. This may be assumed as being the case at approximately 30 single values for each mean. But it is only in exceptional cases that there will be so many replications, and as a rule there will be considerably fewer. Wenzl (1) and Pätau (4) have pointed out on several occasions that the check procedure, irrespective of whether the Z value equals the threshold value of 2, can no longer be supported because in practically all cases the number of single observations is too low, so that it is not possible to make a sufficiently accurate statement on the probability of the existence of a significant difference obtained from means according to the Z method. For the statistical treatment of small series of measurements, use is made for this reason of the t-method worked out by Gosset, which makes allowance for the number of replications. The t-method is based on the assumption that the spot checks to be assessed all originate from the same basic totality. A check is made to establish how great the probability is for this assumption (zero hypothesis). With the t-procedure, we are, therefore, faced with a formulation of the problem different from that in the case of the classical check procedure of the Z value, for which two basic totalities are compared. The t-value is computed according to the following formula:

$$t = (M_1 - M_2) \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{[\text{sum } (x_1 - M_1)^2 + (\text{sum } x_2 - M_2)^2] (n_1 + n_2)}}$$

Making allowance for the degree of freedom $(m_1 + m_2 - 2)$, the corresponding probability value is derived from the t-table. This value is given as p. In agricultural tests, a difference is normally regarded as being adequately significant when the p-value is less than 5 % ($p = < 0{\cdot}05$). This means that the probability for the zero hypothesis is 5 % or less, and therefore that the two spot checks probably do not originate from the same basic totality.

However, the t-procedure cannot always be readily applied, but only when the deviations of the two spot checks do not vary appreciably but fortuitously, i. e. the deviations are not dependent upon the means. The lack of relationship between mean and deviation can mostly be established when arranging the tests units according to progressive means, or when plotting them. Wenzl points out that the deviations increase proportional to the increasing means

expressed as percentages (intensity of infestation from 0—100 %), and decrease again after reaching a peak. The same applies to values which cannot be expressed as percentages (density of infestation per unit of area or plant unit), and for which there is also a correlation between mean and deviation. In these cases, just as for the t-procedure, the analysis of variance cannot be applied as proved by Wenzl, unless of course the material is converted in a suitable manner by different methods, although this often involves comprehensive calculations. A relatively simple solution is provided by applying the approximation method of Cochran and Cox in order to make comparisons of means, for which there is a correlation between mean and deviation. As described, the quotient D/mD is determined, and this is compared with a corrected threshold value which, e. g. for the 5 %-threshold, is computed as follows:

$$\text{Threshold value } (t') = \frac{m_1^2 t_1 + m_2^2 t_2}{m_1^2 + m_2^2}$$

These t-values for P = 5 % are taken from the t-table making allowance for the degree of freedom.

t_1 is the t-value for n_1 — 1 degree of freedom
t_2 is the t-value for n_2 — 1 degree of freedom

When $n_1 = n_2 = n$, then the corresponding probability for the computed quotient D/mD, will be obtained under n — 1 and not under 2n — 2 degrees of freedom.

The t-procedure and the approximation method of Cochran and Cox are sufficient to compute numerical data obtained in plant protection tests.

For further information, attention is drawn to the comprehensive special literature on biological statistics.

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VI. The reliability of the degree of effectiveness established in the field test

An accurate result need not necessarily be a reliable result. The purpose of carrying out tests, however, is to obtain results which can be reproduced, i. e. which can be taken as reliable when the tests are repeated in the following year and at other locations. After all, these results are expected to allow recommendations to be made with as wide a range of validity as possible.

Field workers are not satisfied with the knowledge how a certain control measure acts under the conditions prevailing in one particular year and at a certain location, but they wish to know what is valid for an average number of yields in the whole range of application concerned.

The problem of reliability centres on the question of the annual error. This annual error is the most difficult and at the same time the characteristic error of the field test. This error is not known in any other test procedures. Each attempt to determine the magnitude of the annual error by making an accurate quantitative assessment of the conditions governing the toxic effect for the particular year, meets with the unsurmountable difficulty firstly of being able at all to register all conditions and secondly to calculate a value for them. It is not possible to find a common denominator for such numerous influential factors as wind, rain, temperature, relative humidity and sunshine, and to attribute to this each year an annual error which could be summarized in a numerical expression.

Whereas the accuracy of a test result and the significance of differences in effect can be checked by methods of computation, a useful standard for assessing the reliability of results is still wanting. The reliability of an established degree of effectiveness and of differences in effect cannot be determined by one test no matter how thoroughly it is carried out and evaluated. Each field test only informs us what was good or bad in the respective test year and at the respective location but not what will be good or bad in the coming year. In fact the prospects of such information being furnished dwindle when the conditions governing the toxic effect during the respective year and at the respective location are less typical than usual. It is the test carried out over a period of several years which shows what is correct as based on the average of the values obtained during these years. This is an unalterable deficiency of each field test, which may not be underestimated because on the other hand setbacks must be anticipated. As soon as conclusions are drawn from the tests of one year on what will be experienced in the coming year, one moves away from reality and begins to speculate.

In order to obtain data supporting or disproving the reliability of test results, the test problem must be examined in different years and under different conditions. This is done by several experimenters cooperating closely with each other according to a common test plant. There should not be any deviations whatsoever from this common plan, not even when a question comprising part of the overall problem is found to be of no interest in one particular section of the test. By examining the test problem in a uniform sector for a period of several years a result is obtained which is generally valid for this sector.

There are four possibilities of improving the reliability of the results:

1. The test problem is examined at the same place for several consecutive years. The combined result is then generally valid but only for the place concerned, and within limitations also for the area where the environmental conditions are the same as those prevailing at the test location.
2. The test problem is examined at several locations in one year. The combined result is generally valid for the whole area but only for the year concerned (collective test).

3. The test problem is examined for several consecutive years at different places, but always at one and the same place each year. The combined result is generally valid for the region in all years, whereby a distinction must be made between the influence of location and time.
4. The test problem is examined for several consecutive years at different places of a region each year. The combined result is valid for the region in all years. The influence of location and time can no longer be separated accurately, whereas the general validity is greater than in 3.

The tests must always be carried out at places characteristic of the region. Furthermore, the weather conditions prevailing during the years when the study is made, should be typical for lengthy periods. The tests should be continued for at least three years. 1. and 3. are primarily applicable to perennial plants, 2. and 4. to annual cultures.

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